A Compendium of Vaccine Adjuvants and Excipients (2nd Edition)

Frederick R. Vogel, Michael F. Powell, and Carl R. Alving

Beginning in the early 20th century, researchers experimented with a wide variety of organic and inorganic compounds including aluminum salts, mineral oil, and killed mycobacteria to improve the immunogenicity of vaccines. These first empirical studies demonstrated the adjuvant activity of many substances, but several products also elicited significant local and systemic adverse reactions that precluded their use in human vaccine formulations. Alum adjuvant, first described in 1926, remains the only immunologic adjuvant used in human vaccines licensed in the United States.

Since the advent of modern immunology twenty years ago, hundreds of natural and synthetic compounds have been evaluated as vaccine adjuvants. After extensive safety and toxicity testing, many of these novel adjuvants have proven to be acceptable for clinical evaluation. During the same time, investigations into the mechanisms of action of adjuvants have increased. Today, a major goal of adjuvant research is to apply the increased understanding of basic immunobiology to adjuvant development. Improved understanding of adjuvant mechanisms of action will provide a basis for the rational selection of adjuvants for use with new vaccines. The purpose of this compendium is to provide a reference for investigators interested in accessing information on the numerous adjuvants available for study, and to foster collaboration between basic and applied vaccine researchers with adjuvant developers. This compendium is extensive but by no means complete. It is our hope that vaccinologists, will find this a useful resource and that it may help to advance adjuvant development as an integral part of a rational vaccine design.

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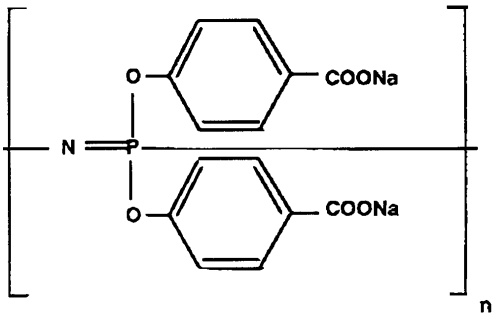
COMPONENT/ADJUVANT NAME: Adjumer™

OTHER NAME(S): PCPP salt; polyphosphazene; polyidi(carboxylatophenoxy)lphosphazene

STRUCTURE:



SOURCE: Synthetic

USES: In the soluble form as an adjuvant for parenteral formulations. In the crosslinked form as a microsphere hydrogel for mucosal formulations

APPEARANCE: Solid: beige to off white powder. Aqueous solution: clear, colorless liquid

MOLECULAR WEIGHT: >700,000 daltons

RECOMMENDED STORAGE: Powder is stable at -20º C. Solution is stable at 4º C.

CHEMICAL/PHYSICAL PROPERTIES: Soluble in aqueous alkali solutions: ionically cross-linkable in aqueous media when treated with salts of di- or trivalent cations;

INCOMPATIBILITY: Precipitates at approximately pH 6.5 or lower. Will cross-link in the presence of di- or trivalent cations

SAFETY/TOXICITY: Has been evaluated in human phase I and II clinical trials. Adjumer™ has been well tolerated with no adverse reactions reported. Drug Master. File submitted to FDA

ADJUVANT PROPERTIES: Induces a sustained antibody response in mice after a single parenteral immunization. Antibody responses include antigen specific IgG1 and IgG2a. Sustained IgG and IgA responses are also induced in mice after mucosal immunization.

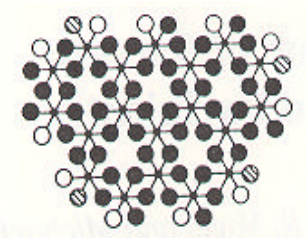
- Payne, L.G. *et al*., 1995, Water soluble phosphazene polymers for parenteral and mucosal vaccine delivery, in: Vaccine Design, M.F. Powell and M.J. Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
- Payne, L.G. *et al*., 1998, PCPP as a parenteral adjuvant for diverse antigens, in: Modulation of the Immune Response to Vaccine Antigens, F. Brown and L.R. Haaheim (Eds.) Developments in Biological Standardization Series, Karger, Basel.
- Payne, L.G. *et al*., 1998, Poly[di(carboxylatophenoxy)phosphazene] (PCPP) is a potent immunoadjuvant for an influenza vaccine. *Vaccine*, 16:92-98.

CONTACT(S): Sharon A. Jenkins, Virus Research Institute, Cambridge, MA, 02138, Ph: 617-864-6232; Fax: 617-864-6334, Email: sjenkins@vrii.com.

COMPONENT/ADJUVANT NAME: Adju-Phos

OTHER NAME(S): Aluminum phosphate gel

STRUCTURE: Amorphous aluminum hydroxyphosphate. A schematic of the unit layer of amorphous aluminum bydroxyphosphate showing the surface hydroxyl, water, and phosphate groups. Key: Al, small closed circle; OH, large closed circle; H_20, open circle; $P0_4$, hatched circle.



SOURCE: Obtained by precipitation. The degree of substitution of phosphate for hydroxyl depends on the concentration of reactants and precipitation conditions.

USES: Human applications: diphtheria, tetanus, and pertussis vaccines. Veterinary vaccine applications.

APPEARANCE: White gelatinous precipitate in aqueous suspension.

MOLECULAR WEIGHT: Not applicable.

RECOMMENDED STORAGE: 4-25° C. Never expose to freezing. Recommended 2 year shelf life.

CHEMICAL/PHYSICAL PROPERTIES: Primary particles have a plate-like morphology and a diameter of 50-100 nm. The isoelectric point is acidic and is inversely related to the degree of substitution of phosphate for hydroxyl. Its high surface area gives it a high adsorptive capacity for antigens. Particle size range of final product 0.5-10,um.

INCOMPATIBILITY: Dissolves in strong bases and acids.

SAFETY/TOXICITY: May cause mild local reactions at the site of injection (erythema and/or mild transient swellings).

- Yamanaka, M. *et al.,* 1992, Pathological studies on local tissue reactions in guinea pigs and rats caused by four different adjuvants, *J. Vet. Med. Sci. 54:685-692.*
- Gupta. *R. K., et al.,* 1993, Adjuvants-A balance between toxicity and adjuvanticity, *Vaccine 11:293-306.*

ADJUVANT PROPERTIES: The surface area, surface charge, and morphology of the amorphous aluminum hydroxyphosphate are major factors in its adjuvant characteristics. The use of aluminum adjuvants is accompanied by stimulation of IL-4 and stimulation of the T-helper-2 subsets in mice, with enhanced IgG1 and IgE production. Properties are described in:

- Seeber, *S., et al.,* 1991, Predicting the adsorption of proteins by aluminum-containing adjuvants, *Vaccine 9:201-203.*
- Shirodkar, *et al.,* 1990, Aluminum compounds used as adjuvants in vaccines, *Pharm. Res. 7:1282-1288.*

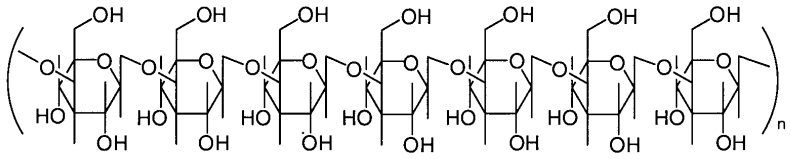
- Seeber, *S. J., et al.,* 1991, Solubilization *of* aluminum-containing adjuvants by constituents of interstitial fluid, *J. Parenteral Sci. Tech. 45:156-159.*
- Gupta, *R. K., et al.,* in: *Vaccine design: the Subunit and Adjuvant Approach*, Powell and Newman (eds.), Plenum, New York, NY (1995), Chapter 8.
- Lindblad, E.B. Aluminum adjuvants, in: *The Theory and Practical Application of Adjuvants*, (Stewart-Tull, ed.), Wiley & Sons, New York, NY (1995), Chapter 2.

CONTACT(S): E. B. Lindblad, Superfos Biosector a/s, DK-2950 Vedbaek, Denmark, Ph: 45 47 38 47 00; Fax: 45 47 38 46 56. Al Reisch, E.M. Sergeant Pulp & Chemical Co., Inc., 6 Chelsea Road Clifton, NJ 07012, Ph: 973-472-9111; Fax: 973-472-5686; E-mail: sergeant@cybernex.net
Stanley Hem, Purdue University, W. Lafayette, IN 47907-1336, Ph: 317-494-145 1; Fax: 317-494-7880.

COMPONENT/ADJUVANT NAME: Algal Glucan

OTHER NAME(S): β-glucan; glucan

STRUCTURE: A linear β-D(1,3)-linked glucopyranose, polymer having a triple-helical conformation.



SOURCE: Produced by an adapted strain of *Euglena gracilis (SRI* strain D86-G) grown heterotrophically in the dark. Obtained from the cytoplasm of the organism by methanol and chloroform extraction. Depyrogenized in hot I N HCI and washed sequentially in pyrogen-free water and pyrogen-free saline.

- Tusé, D., *et al.,* 1992, Production of β-glucan in *Euglena, U.S.* Patent No. 5,084,386.

USES: Administered with antigen for enhancement of both humoral and cell-mediated immunity. β-Glucans exert their immunostimulatory activities by binding to specific β-glucan receptors on macrophages. This ligand-receptor interaction results in macrophage activation and, in certain formulations, promotes antigen targeting.

- DiLuzio, N. R., *et al., 1979,* Evaluation of the mechanism of glucan-induced stimulation of the reticuloendothelial system, *J. Reticuloendothel. Soc. 7:731-742.*
- Czop, J. K., and Austen, K. F, 1985, *A* β-glucan inhibitable receptor on human monocytes: Its identity with the phagocytic receptor for particulate activators of the alternative complement pathway, *J. Immunol. 134:2588-2593.*

APPEARANCE: White, odorless crystalline material. Forms a suspension in aqueous solutions.

MOLECULAR WEIGHT: Highest measured MW = 500,000.

RECOMMENDED STORAGE: Stable to light. Store solid Algal Glucan at room temperature and aqueous suspensions at 4°C. No apparent degradation after storage of aqueous suspension for 24 months at 4°C. Optimal storage conditions are to be determined.

CHEMICAL/PHYSICAL PROPERTIES: Native particulate material is water insoluble. Median particle size 3.7-4.6 µm, with specific gravity of 1.86-2.0 g/cm^3. Purified preparations contain 0.0001-0.35% phosphorus and 0.12-0.27% nitrogen.

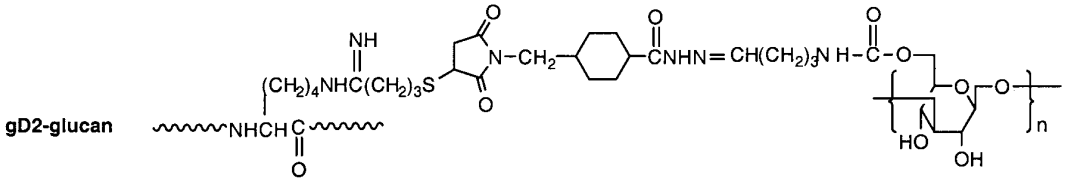
INCOMPATIBILITY: Alkaline pH disrupts the triple-helical conformation.

SAFETY/TOXICITY: In preclinical studies, Algal Glucan has been intravenously administered at doses up to 25 mg/kg body weight and was well tolerated. Human clinical trials of β-glucans isolated from either plants or microorganisms indicate the feasibility of administering these compounds to humans without toxicity. Glucan particles bioerode over time in a physiological environment.

- Mansel, P. W. *A., et al., 1975,* Macrophage-mediated destruction of human malignant cells in vivo, J. *Nad. Cancer Inst. 54:571-580.*

- Okarnura, K., *et al.*, 1986, Clinical evaluation of *Schizophyllon* combined with irradiation in patients with cervical cancer, *Cancer 58:865-872.*
- Chihara, G., *et al.*, *1989,* Lentinan as a host defense potentiator (HDP), *Int.* J. *Immunother, 4:145-154.*
- Ostroff, G. R., *1994,* Future therapeutic applications of Betafectin, a carbohydrate-based immunomodulator, The Second Annual Conference on Glycotechnology.

ADJUVANT PROPERTIES: Algal Glucan, a nonantigenic carbohydrate adjuvant, enhances both humoral and cell-mediated immunity to oligopeptides in experimental animal models. Mice immunized twice by coadministration of herpes virus glycoprotein D (gD2) higher in and 100,ug Algal Glucan produced anti-gD2 antibodies that were significantly higher in titer and persisted longer ($p<0.01$) than those in animals injected with gD2 alone. Similarly, immunization of mice with either gD2- or HIV-1-gp120 with Algal Glucan added as an adjuvant heightened the antigen-specific response of splenic lymphocytes. Finally, antibody titers in C3H/HeJ mice injected with the gD2-β-glucan conjugate were significantly ($p<0.05$) higher than those in animals immunized by coadministration of viral protein and particulate β-glucan.



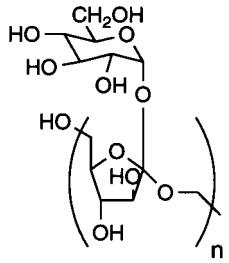
gD2-β-Glucan conjugate

- Mohagheghpour, N., *et al.*, 1992, Adjuvant activity of an algal glycan, VIII International Conference on AIDSAII STI) World Congress, Amsterdam.
- Mohagheghpour, N., *et al.*, 1995. Glucans as immunological adjuvants. In *Immunobiology of Proteins and Peptides VIII* (M.Z. Atassi and G.S. Bixler, Eds.) Plenum Press, New York, pp. 13-22.

CONTACT(S): Dr. Nahid Mohagheghpour, SRI International, Menlo Park, CA 94025, Ph: 650-859-3516; Fax: 650-859-3342. Also: Richard McIntosh, Genesis Technology Group, Inc., Cambridge, MA, Ph: 617-576-6610; Fax: 617-876-4002.

COMPONENT/ADJUVANT NAME: Algammulin

OTHER NAME(S): Gamma inulin/alum composite adjuvant



STRUCTURE: See entries under gamma inulin and Alhydrogel for primary materials. Inulin is crystallized in presence of Alhydrogel suspensions and transformed to gamma inulin at 37° C to form electron-dense ovoids that both adsorb antigen and activate complement.

- Cooper, *R D.,* and Steele, E. J., 1991, Algammulin: A new vaccine adjuvant comprising gamma inulin particles containing alum, *Vaccine* 9:351-357.

USES: Included in adjuvant formulations as a primary adjuvant.

APPEARANCE: Milky white, nonviscous aqueous suspension, easily resuspended. Supplied at 50 mg/mL, sterile and pyrogen-free.

MOLECULAR WEIGHT: See entries under gamma inulin and Alhydrogel.

RECOMMENDED STORAGE: 2-8'C; maintain in aqueous medium. Do not freeze or heat over 45'C.

CHEMICAL/PHYSICAL PROPERTIES: See entries under gamma inulin and Alhy drogel for primary materials. Algammulin is stable for years under recommended storage. Unstable below pH 2 and above pH 10. Virtually insoluble at 37'C.

INCOMPATIBILITY: Degraded in strong acid. Adjuvants containing aluminum hydroxide gel may be incompatible with phosphate or anionic detergents.

SAFETY/TOXICITY: Nonpyrogenic, nonantigenic, and of very low toxicity in experi- mental animals and a Phase I clinical trial. Biodegradable to simple sugars and aluminum hydroxide gel. Large intravenous doses can cause acute complement-activation shock similar to that sometimes found in renal dialysis patients. Dissolved inulin is pharmacologically inert and is registered for human use; alum is also approved for human use.

ADJUVANT PROPERTIES: Expected to stimulate immune responses by causing liga tion of leukocyte-surface complement receptors (CR) via known biochemical mechanisms, thus placing the antigen close to activated leukocytes. Addition of Algarnmulin is known to enhance both humoral and cell-mediated immunity from either Thl or Th2 pathways, depending on the weight ratio of inulin to Alhydrogel.

- Cooper. P. *D. ,et al.*. 1991,The adjuvanticity of Algammulin, a new vaccine adjuvant, *Vaccine* 9:408-415.
- Cooper, *P. D., et al.,* 1993, Gamma inulin and Algammulin: *Two* new vaccine adjuvants, in: *Vaccines 93,*

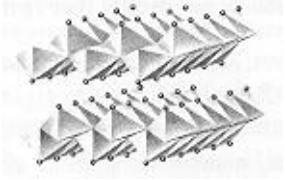
Modem Approaches to New Vaccines Including Prevention of AIDS (H. S. Ginsburg, F Brown, R. M.)
- Chanock, and R. A. Lerner, eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 25-30.
- Cooper, *P. D.*, Chapter 24, this volume.

CONTACT(S): Dr. Peter D. Cooper, Division of Immunology and Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra, A. C. T., Australia 260 1, Ph: 61-6-291-8670; Fax: 61-6-249-2595.

COMPONENT/ADJUVANT NAME: **Alhydrogel**

OTHER NAME(S): Aluminum hydroxide gel; alum

STRUCTURE: Crystalline aluminum oxyhydroxide AIOOH, known mineralogically as boehmite. The structure consists of corrugated sheets of aluminum octahedra.



SOURCE: Obtained by precipitation of aluminum hydroxide under alkaline conditions.

USES: Human applications: diphtheria, tetanus, and pertussis vaccines. Veterinary vaccine applications.

APPEARANCE: White gelatinous precipitate in aqueous suspension.

MOLECULAR WEIGHT: Not applicable.

RECOMMENDED STORAGE: 4-25'C. Never expose to freezing. Recommended 2 year shelf life.

CHEMICAL/PHYSICAL PROPERTIES: Primary particles have a rodlike or fibril morphology and a high surface area. The isoelectric point is 11. Its high surface area gives it a high adsorptive capacity for antigen. Poorly soluble in solutions containing citrate ions. Normal particle size range 0.5-1 Opm.
INCOMPATIBILITY: Dissolves in strong bases and acids.

SAFETY/TOXICITY: May cause mild local reactions at the site of injection (erythemas and/or mild transient swellings).

- Gaarot, P. 0., 1986, Metabolism and possible health effects of aluminurn. *Environ. Health Perspect.* 65:363-441,
- Gupta, *R. K-eral.,* 1993, Adjuvants-A balance between toxicity and adjuvanticity, *Vaccine* 11:293-306.

ADJUVANT PROPERTIES: Alhydrogel is the standard preparations for immunological research on aluminum hydroxide gels. The use of aluminum adjuvants is accompanied by stimulation of IL-4 and stimulation of the T-helper-2 subsets in mice, with enhanced IgG1 and IgE production. Further immunomodu)ation is accomplished by the aluminum content. Properties are described in:

- Shirodkar. S., *et al.,* 1990, Aluminum compounds used as adjuvant in vaccines, *Phar7n. Res.* 7:1282 1288.
- Stewart-Tull, D. E. S., 1989, Recommendations for the assessment of adjuvants (immunomodulators), in: *Immunological Adjuvants and Vaccines* (Gregoriadis, G., Allison, A. C., and Poste. G., eds.), PlenumPress, New York, pp. 213-226.
- Gupta, *R., et al.*. Chapter 8, this volume.

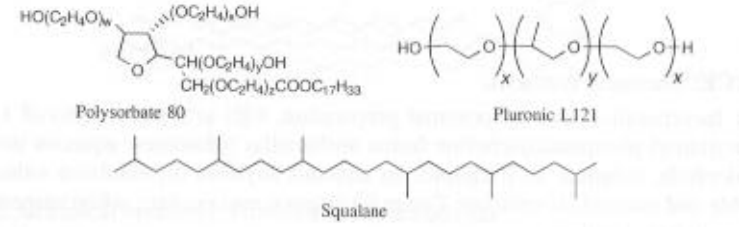
- Seeber, S., *et al.,* 199 1, Predicting the adsorption of proteins by aluminum-containing adjuvants, *Vaccine* 9:201-203.
- Seeber, S. J., *et al.,* 199 1, Solubilization of aluminum-containing adjuvants by constituents of interstitial fluid. *J. Parenteral Sri, Tech.* 45:156-159.
- Hem, S., and White, J. L., Chapter 9, this volume.
- Lindblad, E.B. Aluminum adjuvants, in: *The Theory and Practical Application of Adjuvants*, chapter 2, (Stewart-Tull, ed.), Wiley & Sons, New York, NY (1995).

CONTACT(S): E. B. Lindblad, Superfos Biosector a/s, DK-2950 Vedbaek, Denmark, Ph: 45 47 38 47 00; Fax: 45 47 38 46 56. Al Reisch, E.M. Sergeant Pulp & Chemical Co., Inc., 6 Chelsea Road Clifton, NJ 07012, Ph: 973-472-9111; Fax: 973-472-5686; E-mail: sergeant@cybernex.net. Stanley Hem, Purdue University, West Lafayette, IN 47907-1336, Ph: 317-494-145 1; Fax: 317-494-7880.

COMPONENT/ADJUVANT NAME: Antigen Formulation

OTHER NAME(S): SPT, AF

STRUCTURE: An emulsion of squalane (5%), Tween 80(0.2%), Pluronic L121(1.25%), phosphate-buffered saline pH 7.4, and antigen.



SOURCE: Oil-in-water microemulsion obtained by the microfluidization of the components at reduced temperature.

USES: A vaccine adjuvant vehicle that, when administered with antigen, induces both a cellular and humoral immune response.

APPEARANCE: Homogeneous, white milky liquid.

MOLECULAR WEIGHT: Not applicable.

RECOMMENDED STORAGE: 2-8° C under inert gas. Avoid freezing.

CHEMICAL/PHYSICAL PROPERTIES: A microemulsion comprised of oil droplets of mean diameter around 150-175 nm. Vialed as a 3X formulation, AF is stable for up to 2 years when stored at 5° C, depending on the concentrations of the excipients used as well as the conditions of microfluidization. A uniform dispersion is achieved when diluting 1:3 with aqueous solution prior to administration.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: Pathology and toxicology studies completed in two species, including nonhuman primates. It is well tolerated at doses and schedules that exhibit immune stimulating activity. The safety and potency of the three-component microfluidized formulation has been demonstrated in Phase I/II clinical trials.

ADJUVANT PROPERTIES: Gives good humoral and CTL responses. A potent cytotoxic T cell response was induced when recombinant soluble antigens were injected with AF leading to the destruction of tumor cells or virally infected cells in vitro and in vivo.

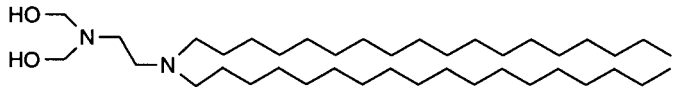
- Raychaudhuri, *et a/.*, 1992, Induction of antigen-specific class I-restricted cytotoxic T cells by soluble proteins in *vivo, Proc. Nad. Acad Sci. USA* 89:8308-8312.

CONTACT(S): Thomas Ryskamp, IDEC Pharmaceuticals Corporation, San Diego, CA 92121, Ph:619-550-8500; Fax:619-550-8750; Internet: tryskamp@idec.com.

COMPONENT/ADJUVANT NAME: Avridine®

OTHER NAME(S): *N,N*-dioctadecyl-*N',N'*-bis(2-hydroxyethyl) propanediamine; CP20,961

STRUCTURE:



SOURCE: Chemical synthesis.

USES: Incorporation into a liposomal preparation, e.g., at a molar ratio of 1:2 Avridine:dimyristoyl phosphatidy1choline forms unilamellar liposomes; aqueous suspensions from alcoholic solution; in Intralipid, an aqueous soybean oil emulsion vehicle; other vegetable and mineral oil vehicles; Tween 80 dispersions in saline; saline suspension with alum-precipitated antigen.

APPEARANCE: White powder.

MOLECULAR WEIGHT: 667.17

RECOMMENDED STORAGE: Store as a powder at room temperature.

CHEMICAL/PHYSICAL PROPERTIES: Very insoluble in water, exhibits waxy properties at temperatures below 39° C; good solubility in absolute ethanol.

INCOMPATIBILITY: None known.

SAFETY/TOXICITY: Intranasal administration to humans induces interferon in nasal secretions and protection against rhinovirus challenge; injection site irritation model for adjuvant arthritis in Lewis rats; antitumor properties in rodent tumor models.

- Niblack,J.F., 1977, Studies with low molecular weight inducers of interferon in man, Toxicol. *Rep.Biol. Med.* 35:528-534.
- Waldman, R. H., and Ganguly, R., 1978, Effect of CP-20,961, an interferon inducer. on upper respiratory tract infections due to rhinovirus type 21 in volunteers, J. *Infect. D*is. 138:531-535.
- Chang, Y. H., et al., 1980, Adjuvant polyarthritis. IV. Induction by a synthetic adjuvant: Immunologic, histopathologic, and other studies, Arthritis *Rheum. 23*:62-71.

ADJUVANT PROPERTIES: Humoral and cellular immunity, proliferation of B and T lymphocytes, protective immunity, activation of macrophages, induction of interferon, enhancement of mucosal immunity when administered orally/enterically with antigen, adjuvanticity with a variety of antigens, induction of IgG2a and IgG2b isotypes.

- Niblack, J. F, *et al.*, 1979, CP-20,96 1: A structurally novel, synthetic adjuvant, J. Reticuloendothel. Soc. 26(Suppl.):655-666.
- Kraaijeveld, C. A., *et al.*, 1982, Enhancement of delayed-type hypersensitivity and induction of interferon by the lipophilic agents DDA and CP-20,961, Cell. Immunol. 74:277-283.

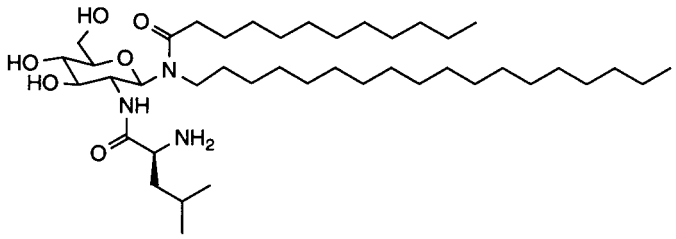
- Jensen, K. E., 1988, Synthetic adjuvants: Avridine and other interferon inducers. in: *Advances in Carriers and Adjuvants for Veterinary Biologics* (R. M. Nerwig, P. M. Gough, M. L. Kaeber, and C. A. Whetstone, eds.), Iowa State University Press, Ames, pp. 79-89.
- Anderson, A. O., *et al*., 1987, Studies on anti-viral mucosal immunity with the lipoidal amine adjuvant Avridine, Adv. *Exp. Med. Biol.* 216B: 1781-1790.

CONTACT(S): Oksana K. Yarosh, VIDO, Saskatoon, Canada S7N OWO, Ph: 306966-7465, Fax: 306-966-7478; E mail: yarosh@&ask.usask.ca. Huw Hughes, M6 Pharmaceuticals, Inc., New York, NY 10701, Ph: 212-308-7200 ext. 14, E-mail: 74577.345@ compuserve.com.

COMPONENT/ADJUVANT NAME: BAY R1005

OTHER NAME(S): N-(2-Deoxy-2-L-leucylamino-β-D-glucopyranosyl)-N-octadecyldodecanoylamide hydroacetate

STRUCTURE:



SOURCE: Chemical synthesis. Provided as the acetate salt.

- Lockhoff, 0., 1991. Glycolipids as immunomodulators: Synthesis and properties, *Angew. Chem. Int. Ed. Engl. 30:1611-1620.*

USES: Primary adjuvant.

APPEARANCE: White lyophilizate.

MOLECULAR WEIGHT: 726.1 + Acetate 60.1

RECOMMENDED STORAGE: Store at 2-8C in airtight containers.

CHEMICAL/PHYSICAL PROPERTIES: Slightly hygroscopic. No polymorphism detected. Chemically stable to air, light, at temperatures up to 50° C, and in aqueous solvents at pH 2-12 at ambient temperature. Amphiphilic molecule, forms micelles in aqueous solution. Formation of translucent liposomal dispersion by ultrasonic treatment.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: Exploratory studies in rats (doses 2.5, 25, or 100 mg/kg body weight) on acute i.p. toxicity according to "OECD Guidelines for Testing Chemicals, No. 401": no deaths, LD5o > 100 mg/kg b.w. subacute i.v. toxicity in rats (doses 2.5, 25, or 100 mg/kg body weight) for 14 days with subsequent 4-week follow-up period to test reversibility of possible effects: no-effect level at 2.5 mg[kg b.w.

ADJUVANT PROPERTIES: BAY R1005 in combination with purified virus vaccines or subunit vaccines led to increased protection of virus-challenged mice. Preclinical trials in other animal species (pig, sheep, horse) gave comparable results with respect to antibody production. The increase in antibody synthesis induced by BAY R1005 is specifically dependent on the antigen and is not the result of polyclonal stimulation. BAY R 1005 acts on the proliferation of B lymphocytes as a second signal which has no effect until the antigen acts as a first signal. BAY R 1005 is capable of activating B lymphocytes without the helper function of T lymphocytes. In mice parenteral immunization with recombinant urease mixed with BAY R1005 induced strong Th1 and Th2

responses and thereby elicited better protection against Helicobacter pylori infection than adjuvants which induced a prominent Th2 type response only.

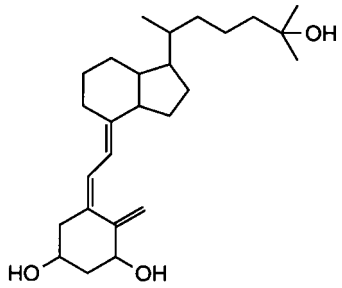
- Stünkel, K. G., *et al.,* 1988, In vitro studies of synthetic glycolipids: A new class of compounds with immunomodulating activity, in: *Leucocyte Activation and Differentiation (J. C.* Mani and J. Dorn, eds.), de Gruyter, Berlin, pp. *421-425.*
- Stünkel, K. G., *et al.,* 1988, Synthetic glycolipids: In vitro characterization of a new class of compounds with immunomodulating properties, Adv. *Biosci. (Oxford) 68:429-437.*
- Stünkel, K. G., *et al.,* 1989, Synthetic glycolipids with immunopotentiating activity on humoral immunity: Evaluation in vivo, *Prog. Leukocyte Biol. 9:575-579.*
- Guy, B., *et al*., 1998. Systemic immunization with urease protects mice against *Helicobacter pylori* infection. *Vaccine* 16:850-856.

CONTACT(S): Dr. 0. Lockhoff, Bayer AG, D-51368 Leverkusen, Germany, Ph: 49-214-30-7958: Fax: 49-214-30-50070. E-mail: oswald.lockhoff.ol@bayer-ag.de

COMPONENT/ADJUVANT NAME: Calcitriol

OTHER NAME(S): lα, 25-dihydroxyvitamin D3; 1,25-di$(OH)_2D_3$; 1,25-DHCC; lα, 25-dihydroxycholecalciferol; 9,10-seco(5Z,7E)-5,7,10(19)-cholestatriene- lα,3β,25-triol

STRUCTURE:



SOURCE: Roche (Nutley, NJ) is the principal supplier to academic researchers. For citations involving the initial identification and methods of preparation seethe *Merck Index* (Merck and Co., Inc., Rahway, NJ) under the entry for calcitriol.

USES: Promotes the induction of mucosal immunity when incorporated into vaccine formulations.

APPEARANCE: White, colorless powder or crystalline material.

MOLECULAR WEIGHT: 416.65

RECOMENDED STORAGE: Air and light sensitive. Storage in *a dry* inert atmosphere at or below -20° C,

CHEMICAL/PHYSICAL PROPERTIES: Calcitriol is a hydrophobic molecule with limited solubility in water. It is soluble in organic solvents including alcohols. Solutions should be prepared in glass to avoid losses of the compound to plastic surfaces. Melting point: 111-1 15° C. Ultraviolet absorption maximum in ethanol is 264 nm (c = 19,000).

INCOMPATIBILITY: Avoid combining calcitriol with components capable of addition or oxidation reactions involving the conjugated *p* electron system. In particular, components capable of releasing free halogens should be avoided.

SAFETY/TOXICITY: Because calcitriol is the active form of vitamin D it should not be given to patients with hypercalcemia. Safety, toxicity, known metabolites, and dosage data are summarized under the entry for Rocaltrol in the *Physicians Desk Reference (Medical* Economics Data Production Co., Montvale, NJ).

ADJUVANT PROPERTIES: The incorporation of calcitrol (0.1-1.0 µg) directly into vaccine formulations containing protein or polysaccharide antigens promotes the induction of both systemic and common mucosal immune responses. Hormone modulation with eliminates the need to apply immunizing agents to mucosal surfaces for induction of secretory antibodies.

- Daynes, R. A., *et al*., 1994, Cytokine modulation in vivo with vitamin D3: Promotion of common mucosal immunity following a standard subcutaneous vacination, *FASEB J*. 8:A283.

- Danyes, R.A., and Araneo, B.A., 1994, The development of effective vaccine adjuvants employing natural regulators of T-cell lymphokine production in vivo, Ann. N.Y. Acad. Sci. 730:144-161.
- Daynes, R. A., *et al*., 1995, Steroids as regulators of the mammalian immune response, J. Invest. Derm. (in press).

CONTACT(S): Raymond A. Daynes, Department of Pathology, University of Utah Medical Center, Salt Lake City, UT 84132, Ph: 801-582-3013; Fax: 801-581-8946.

COMPONENT/ADJUVANT NAME: Calcium Phosphate Gel

OTHER NAME(S): Calcium phosphate

STRUCTURE: Hydrated calcium phosphate gel.

SOURCE: Precipitated by mixing soluble calcium and phosphate salts under carefully controlled conditions.

USES: Calcium phosphate has been used as adjuvant in vaccine formulations against diphtheria, tetanus, pertussis and poliomyelitis. It has also been used for adsorption of allergenic extracts for hyposensitization of allergic patients.

APPEARANCE: White gelatinous precipitate in aqueous suspension.

MOLECULAR WEIGHT: n/a

RECOMMENDED STORAGE: 4-25° C. Never expose to freezing.

CHEMICAL/PHYSICAL PROPERTIES: Adsorbs soluble antigens and presents them in a particulate form to the immune system. Normal particle size range from 0.5-15 µm.

INCOMPATIBILITY: Maintain neutral pH.

SAFETY/TOXICITY: Calcium phosphate adjuvant contains no components that are not natural constituents of the body and is very well tolerated.

- Gupta, R. K. *et al*., 1993, Adjuvants - A balance between toxicity and adjuvanticity. *Vaccine*, 11: 293-306.

ADJUVANT PROPERTIES: Properties are described in:

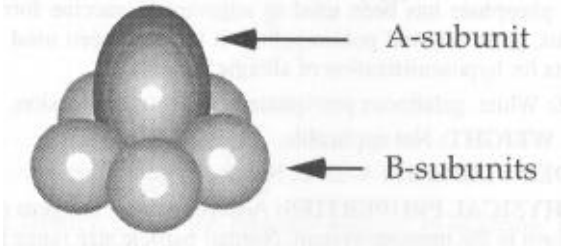
- Relyveld, E. H., 1986, Preparation and use of calcium phosphate-adsorbed vaccines. *Develop. Biol. Stand*., 65: 131-136.
- Relyveld, E. H., *et al*., 1985, Calcium phosphate adjuvanted allergens. *Annals of Allergy*, 54: 521-529.
- Gupta, R., 1995, Adjuvant properties of aluminum and calcium compounds. in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): E. B. Lindblad, Superfos Biosector a/s, DK-2950 Vedbaek, Denmark, Ph: 45 47 38 47 00, Fax: 45 47 38 46 56, Also: Al Reisch, Sargeant, Inc., Clifton, NJ 07012, Ph: 201-472-9111; Fax: 201472-5636.

COMPONENT/ADJUVANT NAME: Cholera holotoxin (CT) and Cholera toxin B subunit (CTB)

OTHER NAME(S): CT; CTB subunit; CTB

STRUCTURE: The CT protein consists of an AB_5-complex that is composed of one enzymatically active A1-subunit which is linked to a pentamer of CTB subunits via the CTA2-fragment. The crystallographic structure for CT has been resolved.



SOURCE: Bacterial protein produced by *Vibrio cholerae* . CTB is the toxoid lacking the A-subunit. Recombinant CTB is available. Mutant holotoxins with partial or no enzymatic activity of the A1-subunit or no receptor-binding ability of the CTB have been produced and have become important tools to identify and explore mechanisms of immune modulation. Suppliers include: List Biological Laboratories, Campbell, CA ; Sigma Chemical Co. St Louis, MO; Swedish National Vaccine Company., Stockholm, Sweden.

USES: CT is the prototype for an ADP-ribosylating bacterial toxin. It binds with high affinity via the CTB to its receptor ganglioside GM1, present on most mammalian cells. It is enzymatically active through its A1-subunit. CT is together with *E. coli* heat labile toxin (LT) perhaps the best studied and most effective mucosal adjuvant yet described for experimental use. Both molecules also have prominent systemic adjuvant effects. Most investigators agree that the immunomodulating effect of CT is strongest when the ADP-ribosylating ability is intact. However, mutant toxins have demonstrated that significant immunomodulation can be achieved by molecules that are enzymatically inactive. Thus, the adjuvant effect of CT is composed of at least two factors; one ADP-riboyltransferase dependent effect and one effect that can be ascribed the AB_5 complex. By contrast, CTB is relatively inefficient as an adjuvant, while it efficiently acts as a carrier/delivery system for other antigens genetically fused or chemically conjugated to CTB. In the murine models such vectors, however, have a stronger propensity for induction of tolerance rather than active immunity following mucosal administration. In humans CTB is a good carrier and highly immunogenic, whereas its tolerogenic effect in humans is unknown. LT but not CT has been tested as adjuvant in humans, albeit toxic effects appeared to have been encountered. CT or mutant derivatives of CT are most frequently used in soluble form simply by admixing with the unrelated protein antigen or, more effective, as chemical conjugates with unrelated protein antigen.

APPEARANCE: White lyophilized powder.

MOLECULAR WEIGHT: CT is 86 kDa ; consisting of the A1-subunit 23kDa, the CTA2 5kDa and five subunits of CTB 11 kDa .

RECOMMENDED STORAGE: Store CT as a lyophilized powder under low humidity at 4°C. After reconstitution with water store CT at - 70C and CTB at 4°C.

CHEMICAL/PHYSICAL PROPERTIES: Good water solubility at neutral pH.

INCOMPATIBILTY: None found. Avoid proteases

SAFETY: CTB is completely non-toxic and has been used extensively in humans without negative side-effects. CT has not been used as an adjuvant in humans because of its toxic effects, even in doses lower than 5 µg.

ADJUVANT PROPERTIES: The mechanism/s responsible for the adjuvant function is not completely understood. Both humoral and cell-mediated immunity, including CTL responses, are known to be greatly augmented by CT-adjuvant. Thus, MHC class I and II restricted responses are enhanced. Some investigators argue that $CD4^+$ T cell differentiation in response to CT-adjuvant is skewed towards a Th2-type, while most evidence support that both Th1 and Th2-type of responses are enhanced. CT exerts immunomodulating effects on T cells, B cells as well as antigen-presenting cells (APC). Which of these effects that are critical for the adjuvant function is at present unknown. However, many observations advocate an effect on the APC, possibly by up-regulation of the expression of co-stimulatory molecules such as CD86, to be important. CTB lacks immunoenhancing effects after oral or intravenous administration, but may augment humoral responses after intranasal and intravaginal administration by acting as an efficient carrier/delivery system.

- Giuliani, M.M., G. Del Giudice, V. Giannelli, G. Dougan, G. Douce, R. Rappuoli, and M. Pizza. 1998. Mucosal adjuvanticity and immunogenicity of LTR72, a novel mutant of escherichia coli heat-labile enterotoxin with partial knockout of ADP- ribosyltransferase activity [In Process Citation]. *J Exp Med* 187(7):1123.
- Lycke, N. 1997. The mechanism of cholera toxin adjuvanticity [In Process Citation]. *Res Immunol* 148(8-9):504.
- Merritt, E.A., and W.G. Hol. 1995. AB5 toxins. *Curr Opin Struct Biol* 5(2):165.
- Rappuoli, R., and M. Pizza. 1991. Structure and evolutionary aspects of ADP-ribosylating toxins. *In* Sourcebook of bacterial protein toxins. J.E.A.a.J.H. Freer, editor. Academic Press, London. 1.
- Spangler, B.D. 1992. Structure and function of cholera toxin and the related Escherichia coli heat-labile enterotoxin. *Microbiol Rev* 56(4):622.
- Sun J, et al., 19 , Cholera toxin B subunit: an efficient transmucosal carrier-delivery system for induction of peripheral immunological tolerance. Proc. Natl. Acad. Sci. USA 1994 ;91:10795.
- Zhang, R.G., *et al*., 1995. The three-dimensional crystal structure of cholera toxin. *J Mol Biol* 251(4):563.

CONTACT(S): Nils Lycke , University of Göteborg, S-413 46 Göteborg Sweden.Ph: 46-31-604936, FAX: 46-31-827647 e-mail: nils.lycke@microbio.gu.se. Also John G. Nedrud, Ph.D., Associate Professor of Pathology, Department of Pathology Biomedical Research Building, Room 919 Case Western Reserve University 10900 Euclid Avenue, Cleveland, OH 44106, USA. Ph.: (216) 368-1281, Fax:(216) 368-1300/368-1357/368-0495, e-mail: jgn3@po.cwru.edu

COMPONENT/ADJUVANT NAME: Cholera toxin A1-subunit-ProteinA D-fragment fusion protein

OTHER NAME(S): CTA1-DD gene fusion protein

STRUCTURE: The CTA1-DD protein consists of a genetically engineered fusion between genes encoding the cholera toxin A1-subunit and a dimer of a synthetic analogue of the D-fragment of *Staph. aureus* proteinA. The CTA1 moiety is an enzymatically active, ADP-ribosylating moiety, while the DD-dimer binds to immunoglobulins of all isotypes. CTA1-DD binds to both Fc- and Fab-fragments. The ADP-ribosylating ability, on a molar level, was roughhly 50% of that of intact CT. Of note, neither the CTA2 nor CTB subunits of the cholera holotoxin are present in the fusion protein.

SOURCE: The CTA1-DD plasmid is expressed in *E. coli* and the protein is purified to high purity on an IgG-column. Mutant CTA1-DD molecules have been constructed that do not ADP-ribosylate nor bind to Fc-fragments of immunoglobulin.

USES: The CTA1-DD fusion protein has proven equivalently potent as an adjuvant to the intact cholera holotoxin (CT) for humoral and cell-mediated immunity. CTA1-DD has successfully been evaluated as a systemic and mucosal adjuvant in mice, but not in humans. After i.p, i.v, intranasal, rectal or intravaginal-, but not oral, administration. in mice 50-500-fold enhancement of specific responses were observed. The CTA1-DD was found to be completely non-toxic, and had retained the adjuvant function of the intact holotoxin. Thus it effectively separates toxicity from adjuvanticity. The fusion protein introduces a completely novel concept in vaccine adjuvant construction/design, namely, cell-specific targeted immunomodulation. CTA1-DD binds to B lymphocytes and is enriched to B cell follicles in the spleen after i.v injection. CTA1-DD, similar to CT, is used in soluble form simply by admixing with the unrelated protein antigen or, more effective, as chemical conjugates with unrelated protein antigen. The CTA1-DD can host immunogenic peptides inserted between the two moieties and acts then as a powerful targeted delivery and immunoenhancing vector.

APPEARANCE: A clear solution in 0.2M HAc-buffer at pH 3.

MOLECULAR WEIGHT: CTA1-DD is 37kDa ; consisting of the A1-subunit 23kDa and the dimer of D, each 7 kDa.

RECOMMENDED STORAGE: In soluble form in 0.2M HAc-buffer pH 3 at 4°C. Do not freeze. Stability in the frige at 4°C with unaltered activity lasts about 2-3 months. Solubility and stability are reduced at neutral pH.

CHEMICAL/PHYSICAL PROPERTIES: Water solubility at neutral pH is relatively good, but precipitation may occur. Mutants with increased solubility has been constructed.

INCOMPATIBILTY: Sensitive to proteases.

SAFETY: CTA1-DD is non-toxic in mice. It has not been tested in humans.

ADJUVANT PROPERTIES: The CTA1-DD adjuvant is targeted to B lymphocytes, both memory and naive cells. CTA1-DD is a powerful systemic and mucosal adjuvant. The mechanism for its adjuvant function is at present not completely understood. Studies of CT and CTA1-DD have revealed comparable adjuvant properties, despite that CTA1-DD has a very limited scope of binding to immunocytes compared to CT. CTA1-DD

augments the expression of co-stimulatory molecules on antigen-presenting cells (APC), i.e. B cells. Binding or interactions with dendritic cells and macrophages have not been found. $CD4^+$ T cell priming and CTL activity have been found to be augmented.The CTA1-DD adjuvant has direct effects on B cell activation and differentiation, but direct effects on T cells has not been observed. Increased specific IgM responses have been observed in T cell deficient nu/nu mice.

- Lycke, N. 1997. The mechanism of cholera toxin adjuvanticity [In Process Citation]. *Res Immunol* 148(89):504.
- Ågren, L., *et al*., 1997, A genetically engineered non-toxic vaccine adjuvant that combines B cell targeting with immunomodulation by cholera toxin A1 subunit. *J. Immunol* 158:3936.
- Ågren, L., *et al*.,1998, A novel concept in mucosal adjuvanticity: The CTA1-DD adjuvant is a B cell targeted fusion protein that incorporates the enzymatically active cholera toxin A1 subunit. *Immunol & Cell Biology* 76:280-287.

CONTACTS: Nils Lycke , University of Göteborg, S-413 46 Göteborg Sweden.Ph: 46-31-604936, Fax: 46-31-827647 e-mail: nils.lycke@microbio.gu.se.

COMPONENT/ADJUVANT NAME: CRL1005

OTHER NAME(S): Block Copolymer P1205

STRUCTURE: ABA block polymer with mean values of $x = 8$ and $y = 205$.

SOURCE: Linear chain polymers are synthesized by condensation of propylene oxide and ethylene glycol initiator in the presence of a cesium salt catalyst to form polyoxypropylene chain, followed by condensation of ethylene oxide on either end of the chain. Individual polymeric species of triblock nonionic block copolymers result from controlled synthesis of chains with pre-determined length.

USES: A component of adjuvant formulations. The formulation is customized for particular uses. The water-in-oil emulsion typically contains 80% saline, and 20% oil phase consisting of squalene and span 80. The copolymer is added to the aqueous phase in amounts sufficient for the required dose. It acts as both an adjuvant and stabilizer. The water-in-oil-in-water (w/o/w) multiple emulsion is prepared similarly with the addition of an outer aqueous phase.

APPEARANCE: Clear, colorless to slightly yellow, viscous liquid.

MOLECULAR WEIGHT: Approx. 12.5 kD.

RECOMMENDED STORAGE: CRL1005 can be stored in tight amber glass containers with minimum headspace at 4° C for 2-3 years. Aqueous solutions (<10% w/v) stored at 4° C.

CHEMICAL/PHYSICAL PROPERTIES: CRL1005 is soluble in neutral or near neutral (pH 5.5 - 8) aqueous buffers at temperatures <4° C up to 10% (w/v). Above 4° C CRL1005 coalesces and forms large, stable micelle-like structures 250-300 nm. in diameter.

INCOMPATIBILITY: CRL1005 is compatible with aqueous buffering systems and can incorporate into the oil phase of oil-based emulsion vehicles. Compatible with a wide number of antigens, but more effective with intact proteins than peptides.

SAFETY/TOXICITY: Aqueous polymer suspensions of CRL1005 evaluated in rodent species in conjunction with influenza HA vaccines, with no adverse safety events noted. Microemulsions of CRL1005 with squalene evaluated in rodent species, with no adverse effects. Data not yet available for human safety.

ADJUVANT PROPERTIES: CRL1005 forms microparticulate structures that can bind a variety of antigens via a combination of hydrophobic interactions and surface charge. Available data suggest block copolymers influence epitope recognition and induce protective (e.g., IgG2a) antibody subclasses. Some formulations, particularly multiple emulsions, have potential as mucosal delivery vehicles. Data on cellular immunity not available.

- Hunter, R. L. *et al*., 1981, Studies on the adjuvant activity of nonionic block polymer surfactants. 1. The role of hydrophile-lipophile balance. *J. Immunol*., 127:1244-1250.
- Hunter, R. L. *et al*., 1991, Adjuvant activity of nonionic block copolymers, IV. Effect of molecular weight and formulation on titer and isotype of antibody. *Vaccine*, 9:250-256.
- Takayama, K. *et al*. 1991, Adjuvant activity of nonionic block copolymers, V. Modulation of antibody isotype by lipopolysaccharides, Lipid A and precursors. Vaccine, 9:257-265.

- Kalish, M. L. *et al*., 1991, Murine IgG isotype responses to the Plasmodium cynomolgi circurnsporozoite protein (NAGG)5. I. Effects of carrier, copolymer adjuvants, and nontoxic LPS on isotype distribution. Infect. Immun., 59:2750-2757.
- van de Wijgert, J. H. H. M. *et al*., 1991. Immunogenicity of *Streptococcus pneumoniae* type 14 capsular polysaccharide: influence of carrier and adjuvants on isotype distribution. Infect. Immun., 59:2750-2757.
- ten Hagen, T. L. M. *et al*., 1993, The role of adjuvants in the modulation of antibody specificity and induction of protection by whole blood-stage *Plasmodium yoelii* vaccines. J. Irnmunol., 151:7077-7085.
- Brey, R. Development of vaccines based on formulations containing nonionic block copolymers, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York (1995).

CONTACT(S): Vaxcel Corporation, GA 30092 Ph: 404-447-9330; Fax: 404-447-8875.

COMPONENT/ADJUVANT NAME: Cytokine-containing Liposomes

OTHER NAME(S): Cytokine-containing Dehydration Rehydration Vesicles.

STRUCTURE: This is a dehydration-rehydration liposome composed of phosphotidylchohne (PC) and cholesterol in a 1:1 molar ratio and recombinant cytokines. The following cytokines have been tested IL-lα, IL-1β, IL-6, TNFα, and interferon-γ.

SOURCE: The lipids are purchased from Avanti Polar-Lipids, Inc. Alabaster, AL. Cytokines are purchased from commercial sources and should not be contaminated with endotoxin.

USES: Induces both cellular and humoral immunity.

APPEARANCE: Cloudy suspension when in solution or white powder when dried.

MOLECULAR WEIGHT: See below for physical properties.

RECOMMENDED STORAGE: Prepared immediately before use, but may be stored at 4 'C.

CHEMICAL/PHYSICAL PROPERTIES: The size of the liposomes has been determined to be between 50 and 300 nm as determined by electron microscopy.

INCOMPATIBILITY: None known.

SAFETY/TOXICITY: Multilamellar liposomes are in clinical trials in humans.

ADJUVANT PROPERTIES:

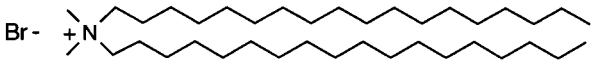
- Murray, J. L. *et al.*, 1989. Phase I trial of liposomal muramyl tripeptide phosphatidylethanolamine in cancer patients, *J. Clin. Oncol.*, 7:1915-1925.
- Fidler, I. J., 1988. Targeting of immunomodulators to mononuclear phagocytes for therapy of cancer, *Adv. Drug Deliv. Res.*, 2:69-83.
- Fogler, W. E. *et al.*, 1985, Distribution and fate of free and liposome-encapsulated [^{3}H]nor-muramyl dipeptide and [^{3}H]muramyl tripeptide phosphatidylethanolamine in mice, *J. Immunol.*, 135:1372-1377.
- Lopez Berestein, G. *et al.*, 1985, Lipsomal amphotericin B for the treatment of systemic fungal infections in patients with cancer: a preliminary study, *J. Infect. Dis.*, 151:704-710.
- Gregoriadis, G. *et al.*, Liposomes as immunological adjuvants: Antigen incorporation studies, Vaccine, 5:145-151.
- Lachman, L. *et al*, 1995, Cytokine containing liposomes as adjuvants for subunit vaccines, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Lawrence B. Lachman, Ph.D., University of Texas M.D. Anderson Cancer Center, Department of Cell Biology, Houston, TX, 77030, Ph: 713-792-8587; Fax: 713-797-9764; E-mail: lachman@odin.mdacc.tmc.edu

COMPONENTIADJUVANT NAME: DDA

OTHER NAME(S): Dimethy1dioctadecylammonium bromide; dimethy1distearylammonium bromide (CAS Registry Number 3700-67-2).

STRUCTURE:



SOURCE: The chloride analog of DDA is present in materials known as di(hydrogenate tallow)dimethylammonium salts available under various trade names (e.g., Quarternium-18, Adogen 442-110P, Cycloton D261 C/75, etc.). These salts comprise alkyl chains ranging from 12 to 18 carbon atoms with a typical distribution of C12:C14:C16:C18 = 1:4:31:64.

USES: For stimulation immune responses against various antigens and especially delayed type hypersensitivity. Oil-based emulsions in association with liposomes; also as a non-oil emulsion.

APPEARANCE: White, odorless powder.

MOLECULAR WEIGHT: 631

RECOMMENDED STORAGE: 4-20° C. Protect from light.

CHEMICAL/PHYSICAL PROPERTIES: Hydrophilic quaternary amine; positively charged surface-active substance with bromide (optionally chloride) as counterion. Gel-liquid transition temperature of 39.5° C. Poorly soluble in cold water but readily soluble/ dispersible in warm water in which it forms liposomal structures. Soluble in organic solvents.

INCOMPATIBILITY: Complexes are formed with multivalent, negatively-charged molecules (e.g., phosphate) in aqueous phase which might precipitate.

SAFETY/TOXICITY: Parenteral administration of DDA induces a mild inflammatory reaction at the site of injection (swelling and influx of polymorphonuclear neutrophils, macrophages, and lymphocytes). Effective dose range 1-10 mg/kg in small animals, and 0.01-1 mg/kg in large animals. Human trials include:

- Stanfield, J. P. *et al.*, 1973, Single dose antenatal tetanus immunization, *Lancet*, 301: 215-19.
- Chambers, J. D. *et al.*, 1980, Induction of specific transplantation tolerance in man by autoblast immunization, *Blood*, 41: 229-236.

ADJUVANT PROPERTIES: DDA stimulates both humoral and cell-mediated immune responses against a wide range of antigens and in various animal species. Especially delayed-type hypersensitivity reaction are augmented strongly by DDA after administration via subcutaneous for intracutaneous route. Functions as a carrier of antigen by direct binding of antigen, or modification at the oil/water interface.

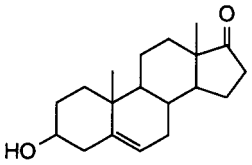
- Hilgers, L. A. Th. and Snippe, H., 1992, DDA as immunological adjuvant, *Res. Immunology*, 143:494-503.
- Snippe, H. and Kraayeveld, C., 1989, The immunoadjuvant dimethyldioctadecylammonium bromide. In: *Immunological Adjuvants and Vaccines* (Gregoriadis, G., Allison, A. C., and Poste, G., eds), Plenum Press, New York, pp. 47-59.

CONTACT(S): L. Hilgers, Solvay S.A., Research & Technology, Central Laboratory, Applied Immunology, Rue de Ransbeek 310, B-1120, Brussels, Belgium, or H. Snippe, University Utrecht, Eijkman-Winkler Laboratorium for Medical Microbiology, 3584 CX Utrecht, The Netherlands. Also, Eastman Kodak Company, Rochester NY 14650, USA, Ph: 716-458-3702; Fax: 716-722-3172. Also: Huw Hughes, M6 Pharmaceuticals, Inc., Yonkers, NY 10701. Ph: 914-476-6799; E-mail: 74577.345@compuserve.com.

COMPONENTIADJUVANT NAME: DHEA

OTHER NAME(S): Dehydroepiandrosterone; 5-androsten-3β-ol-17-one; dehydroisoandrosterone; androstenolone; prasterone; transdehydroandrosterone; DHA

STRUCTURE:



SOURCE: Commercially available from numerous suppliers. For citations involving the initial identification and methods of preparation see the Merck Index (Merck and Co., Inc.; Rahway, N.J.) under the entry for prasterone.

USES: DHEA can be directly incorporated into vaccine formulations (2-10 µg/vaccination in mice, 100 µg/vaccination in dogs) and will enhance antibody formation.

APPEARANCE: White, colorless powder or crystals.

MOLECULAR WEIGHT: 288.4

RECOMMENDED STORAGE: DHEA is relatively stable, however, its double bond is susceptible to both addition and oxidation reactions. Long term storage under dry inert gas at or below -20° C.

CHEMICALIPHYSICAL PROPERTIES: DHEA is a hydrophobic molecule with limited solubility in water. It is soluble in organic solvents including alcohols and dimethylsulfoxide, and only slightly soluble in petroleum ether. Literature melting point varies: dimorphous needles 140-141° C, leaflets 152-153° C (Merck Index) crystals 149-151° C (Steraloids; Wilton, NH).

INCOMPATIBILITY: Do not combine DHEA with components capable of addition or oxidation reactions involving the *p* electron system, including components capable of releasing free halogens.

SAFETY/TOXICITY: Dosages up to 1600 mg per day have been given orally to humans with no adverse reaction.

ADJUVANT PROPERTIES: Administration of DHEA or its sulfate to animals that are immunologically compromised as a consequence of age rapidly restore normal immunologic competence. DHEA can be administered systemically (approximately 4 mg/kg/day) to animals at the time of vaccination, or can be directly incorporated into the vaccine formulation.

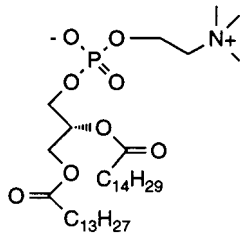
- Araneo, B. A. *et al*., 1993. Reversal of the immunosenescent phenotype by dehydroepiandrosterone: Hormone treatment provides an adjuvant effect on the immunization of aged mice with recombinant hepatitis B surface antigen. *J. Infect. Dis.*167: 830-840.
- Daynes, R. A. and Araneo, B. A., 1992, Prevention and reversal of some age-associated changes in immunologic responses by supplemental dehydroepiandrosterone sulfate therapy. Aging: *Immununol. Infect. Dis*. 3:135-154.
- Araneo, B. A., *et al*., 1993. Administration of DHEA to burned mice preserves normal immunologic competence. *Arch. Surg*. 128: 318-325.

CONTACT: Raymond A. Daynes, Dept. of Pathology, Univ. of Utah Medical Center, Salt Lake City, UT 84132, Ph: 801-581-3013; Fax: 801-581-8946.

COMPONENT/ADJUVANT NAME: DMPC

OTHER NAME(S): Dimyristoyl phosphatidy1choline; sn-3-phosphatidyl choline-1, 2-dimyristoyl; 1, 2-dimyristoyl-sn-3-phosphatidyl choline; (CAS Registry Number 18194-24-6)

STRUCTURE:



SOURCE: Chemical synthesis.

- Walts, A. E., *et al*, 1992, Applications of Biocatalysts in the Synthesis of Phospholipids, in Chirality in Industry, Ed by A. N. Collins, G. N. Sheldrake and J. Crosby. John Wiley & Sons, New York.

USES: Used in the manufacture of pharmaceutical grade liposomes, typically in combination with DMPG and/or cholesterol. Also used in adjuvant systems for vaccine formulations. Applications in novel forms of drug delivery.

APPEARANCE: White,powder.

MOLECULAR WEIGHT: 677.9

RECOMMENDED STORAGE: Keep airtight. Keep out of light. Store at 0-5° C.

CHEMICAL/PHYSICAL PROPERTIES: Amphiphilic solid. Poorly soluble in water. Solubility tends to increase as the pH is lowered within the range pH 3-8. Subject to hydrolysis below pH 3 and above pH 8.

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: Has been used in numerous clinical trials without reported safety/toxicity issues.

ADJUVANT PROPERTIES:

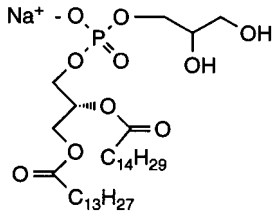
- Alving, C. R., 1993, Immunologic presentation of liposomal antigens. *J. Liposome Research*, 3:493-504.
- Just, M., *et al.*, 1992, A single vaccination with an inactivated hepatitis A liposome vaccine induces protective antibodies after only two weeks. *Vaccine*, 10:737-739.
- Ghick, R., 1994, Liposomal presentation of antigens for human vaccines, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
- Pietrobon, P., 1994, Liposomal design and vaccine development, in: Vaccine Design, M. F. Powell and M. J Newman (eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Tony Newton, Genzyme Pharmaceuticals and Fine Chemicals, Cambridge, MA, 02139, Ph: 617-252-7783; Fax: 617-252-7772.

COMPONENT/ADJUVANT NAME: DMPG

OTHER NAME(S): Dimyristoyl phosphatidylglycerol; sn-3-phosphatidyl glycerol-1, 2-dimyristoyl, sodium salt (CAS Registry Number 67232-80-8); 1, 2-dimyristoyl-sn-3-phosphatidyl glycerol

STRUCTURE:



SOURCE: Chemical synthesis.

- Walts, A. E., et al, 1992, Applications of biocatalysts in the synthesis of phospholipids, in: Chirality in Industry, A. N. Collins, G. N. Sheldrake and J. Crosby, eds. John Wiley & Sons, New York.

USES: Used in the manufacture of pharmaceutical grade liposomes, typically in combination with DMPC and/or cholesterol. Also used in adjuvant systems for vaccine formulations. Applications in novel forms of d d Iiruff ivik

APPEARANCE: White powder.

MOLECULAR WEIGHT: 688.9

RECOMMENDED STORAGE: Keep airtight. Keep out of light. Store at 0-5° C.

CHEMICAL/PHYSICAL PROPERTIES: Amphiphilic solid. Poorly soluble in water. Solubility tends to increase as the pH is lowered within the range pH 3-8. Subject to hydrolysis below pH 3 and above pH 8.

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: Has been used in numerous clinical trials without reported safety/toxicity issues.

ADJUVANT PROPERTIES:

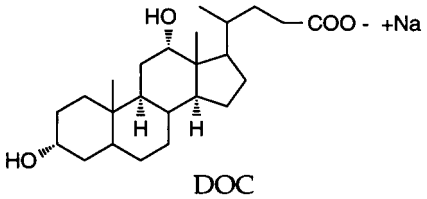
- Alving, C. R., 1993, Immunologic presentation of liposomal antigens. *J. Liposome Research*, 3:493-504.
- Just, M. *et al*., 1992, A single vaccination with an inactivated hepatitis A liposome vaccine induces protective antibodies after only two weeks. *Vaccine*, 10:737-739.
- Ghick, R., 1994, Liposomal presentation of antigens for human vaccines, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
- Pietrobon, P., 1995, Liposomal design and vaccine development, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Tony Newton, Genzyme Pharmaceuticals and Fine Chemicals, Cambridge, MA, 02139, Ph: 617-252-7783; Fax: 617-252-7772.

COMPONENT/ADJUVANT NAME: DOC/Alum Complex

OTHER NAME(S): Deoxycholic Acid Sodium Salt; DOC /$Al(OH)_3$/ mineral carrier complex

STRUCTURE:



DOC

SOURCE: DOC obtained from Sigma Chemicals.

USES: DOC has been used as a detergent. Complex used as adjuvant formulation.

APPEARANCE: White powder, or a clear, colorless solution.

MOLECULAR WEIGHT: 414.6

RECOMMENDED STORAGE: 4° C

CHEMICAL/PHYSICAL PROPERTIES: Gives typical reactions common for all bile acids. Optical rotation $[\alpha]_D20 = + 44 \pm 2°$ [c = 2% (w/v) in water].

INCOMPATIBILITY: Precipitates below pH 6 and in presence of divalent cations, forms gels at temperature below 10° C (reversible).

SAFETY/TOXICITY:

ADJUVANT PROPERTIES: Enhances immune response to membrane proteins.

- Barrett, N. *et al*., 1989, Large-scale production and purification of a vaccinia recombinant-derived HIV-1 gp160 and analysis of its immunogenicity. *AIDS Res. and Human Retroviruses*, 5:157-171.

CONTACT(S): Professor Friedrich Dorner, Ph.D., Immuno AG, Biomedical Research Center, A-2304 Ortha/Donau, Austria, Ph: 43-2212-2701/ext. 300, Fax: 43-2212-2716.

COMPONENT/ADJUVANT NAME: Freund's Complete Adjuvant

OTHER NAME(S): Complete Freund's adjuvant; CIA; FCA

STRUCTURE: Mixture of mineral oil (Marco 52) and emulsifier (Arlacel A [mannide monooleate]) as an emulsion of 85% mineral oil and 15% emulsifier with 500 µg heat-killed and dried *Mycobacterium tuberculosis* per mL of emulsifier mixture.

SOURCE: *M. tuberculosis* grown and adjuvant is manufactured at the Statens Seruminstitut, Copenhagen, Denmark.

USES: The ethics of using Freund's complete adjuvant in animals are at present disputed, due to the profile of severe side-effects.

APPEARANCE: Thick viscous liquid without color.

MOLECULAR WEIGHT: n/a

RECOMMENDED STORAGE: Store at 2-8° C. Do not freeze the final emulsion, as it is disrupted by freezing.

CHEMICAL/PHYSICAL PROPERTIES: Mixing (usually syringe-to-syringe mixing) with an aqueous antigen phase in a 1:1 ratio makes a water-in-oil emulsion (w/o) ready for immunization.

INCOMPATIBILITY: Avoid freezing.

SAFETY/TOXICITY: May cause granulomas and abscesses at the site of injection. May cause arthritis, amyloidosis and allergic reactions. Causes ascites, production in BALB/C mice when injected i.p. with antigen.

- Yamanaka, M. *et al*, 1992, Pathological studies on local tissue reactions in guinea pigs and rats caused by four different adjuvants, *J. Vet. Med. Sci.*, 54:685-92.

ADJUVANT PROPERTIES:

- Freund, J., 1956, The mode of action of immunologic adjuvants. *Adv. Tuberc. Res.*, 7:130-148.
- Herbert, W. J., 1967, Methods for the preparation of water-in-oil, and multiple, emulsions for use as antigen adjuvants; and notes on their use in immunization procedures. In: Handbook of Experimental Immunology, D. M. Weir and Blackwell, Eds., 1207-1217.
- Bomford, R., 1980, The comparative selectivity of adjuvants for humoral and cell-mediated immunity. II. Effect on delayed-type hypersensitivity in the mouse and guinea pig, and cell-mediated immunity to tumor antigens in the mouse of Freund's incomplete and complete adjuvants, Alhydrogel, *Corynebacterium parvum, Bordetella pertussis,* muramyl dipeptide and saponin. *Clin. Exp. Immunol.*, 39:435-441.

CONTACT(S): There are several contacts for CFA (see also Montanide ISA monographs). This product is by: Erik B. Lindblad, Superfos Biosector, DK-2950 Vedbaek, Denmark, Ph: 45 47 38 4700; Fax: 45 47 38 46 56, Also: Al Reisch, Sargeant, Inc., Clifton, NJ 07012, Ph: 201-472-9111; Fax: 201-472-5686.

COMPONENT/ADJUVANT NAME: Freund's Incomplete Adjuvant

OTHER NAME(S): Incomplete Freund's Adjuvant; IFA;FIA

STRUCTURE: Mixture of mineral oil (Marcol 52) and emulsifier (Arlacel A [mannide monooleate]) as an 80% mineral oil, and 15% emulsifier emulsion.

SOURCE: Manufactured by Statens Seruminstitut, Copenhagen, Denmark

USES: Immunization of experimental animals.

APPEARANCE: Thick viscous liquid without color.

MOLECULAR WEIGHT: n/a

RECOMMENDED STORAGE: Store at 2-8° C. Do not freeze the final emulsion, as it is disrupted by freezing.

CHEMICAL/PHYSICAL PROPERTIES: Mixing (usually syringe-to-syringe mixing) with an aqueous antigen phase in a 1:1 ratio makes a water-in-oil emulsion ready for immunization.

INCOMPATIBILITY: Avoid freezing.

SAFETY/TOXICITY: May cause granulomas and abscesses at the site of injection. Induces production of ascites in BALB/C mice when injected i.p. with antigen.

- Yamanaka, M., *et al*, 1992, Pathological studies on local tissue reactions in guinea pigs and rats caused by four different adjuvants, *J. Vet. Med. Sci*., 54:685-92.

ADJUVANT PROPERTIES:

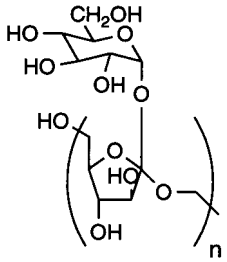
- Herbert, W. J., 1967, Methods for the preparation of water-in-oil, and multiple, emulsions for use as antigen adjuvants; and notes on their use in immunization procedures. Handbook of Experimental Immunology, D. M. Weir and Blackwell, Eds., 1207-1217.
- Bomford, R., 1980, The comparative selectivity of adjuvants for humoral and cell-mediated immunity. 11. Effect on delayed-type hypersensitivity in the mouse and guinea pig, and cell-mediated immunity to tumour antigens in the mouse of Freund's incomplete and complete adjuvants, Alhydrogel, *Corynebacterium. parvum, Bordetella pertussis*, muramyl dipeptide and saponin. *Clin. Exp. Immunol*., 39:435-441.

CONTACT(S): There are several contacts for IFA (see also Montanide ISA monographs). This product is by: Erik B. Lindblad, Superfos Biosector, DK-2950 Vedbaek, Denmark, Ph: 45 47 38 4700; Fax: 45 47 38 46 56, Also: Al Reisch, Sargeant, Inc., Clifton, NJ 07012, Ph: 201-472-9111; Fax: 201-472-5686.

COMPONENT/ADJUVANT NAME: Gamma Inulin

OTHER NAME(S): None.

STRUCTURE: Linear (unbranched) β-D-(2-1) polyfructofuranosyl-α-D-glucose, as particles in the gamma polymorphic configuration. Typically *n* = 50-75.



SOURCE: Dahlia tubers. Obtained by aqueous extraction and crystallization of inulin, followed by adsorptive treatments, recrystallization and conversion to the gamma form at 37° C.

- Cooper, P. D. and Carter, M., 1986, Anticomplementary action of polymorphic "solubility forms" of particulate inulin. Molec. Immunol. 23:895-901.

USES: Highly specific activator of the alternative pathway of complement in vitro and in vivo. Included in adjuvant formulations as a primary adjuvant and also as the immune stimulant when combined as composite particles with alum in the adjuvant Algammulin.

APPEARANCE: Milky white nonviscous aqueous suspension, easily resuspended. Supplied at 50 mg/mL, sterile and pyrogen-free.

MOLECULAR WEIGHT: 8-12 kD. A typical preparation comprises a range of chain lengths corresponding to degrees of polymerization of 50-75 fructose residues.

RECOMMENDED STORAGE: 2-8° C in aqueous medium. Do not freeze or heat over 45° C.

CHEMICAL/PHYSICAL PROPERTIES: Neutral, edible polysaccharide of known primary structure, as ovoids about 1 micron diameter. Stable for years under recommended storage. Unstable below pH 2 and above pH 10. The gamma form is virtually insoluble at 37° C and is essential for biological

INCOMPATIBILITY: Degraded in moderately strong acid.

SAFETY/TOXICITY: Nonpyrogenic, nonantigenic and of very low toxicity in experimental animals, Biodegradable to simple sugars. Large intravenous doses can cause acute complement-activation shock similar to that sometimes found in human renal dialysis patients. Dissolved inulin is pharmacologically inert and is registered for human use.

ADJUVANT PROPERTIES: Expected to stimulate immune responses by causing ligation of leukocyte-surface complement receptors (CR) via known biochemical mechanisms. Addition of gamma inulin is known to enhance both humoral and cell-mediated immunity from both Thl and Th2 pathways. Gamma inulin also has an antitumor action and an effect on natural immunity.

- Cooper, P. D. and Steele, E. J., 1988, The adjuvanticity of gamma inulin. *Immunol. Cell Biol.*, 66:345-352.

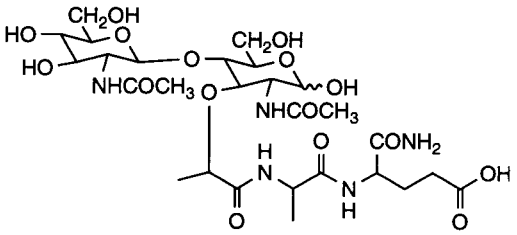
- Cooper, P. D. et al., 1993, Gamma inulin and Algammulin: Two new vaccine adjuvants, in: Vaccines 93, Modem Approaches to New Vaccines Including Prevention of AIDS Ginsburg, H.S., Brown, F., Chanock, R.M., and Lerner, R.A., Eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp.25-30.
- Cooper, P. D., 1995, Vaccine adjuvants based on gamma inulin, in: Vaccine Design, M. F. Powell and M. Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT: Dr. P.D. Cooper, Division of Immunology and Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T., Australia 2601. Ph: 61-6-291-8670; Fax: 61-6-2492595.

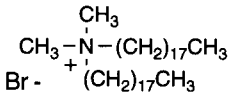
COMPONENT/ADJUVANT NAME: Gerbu Adjuvant

OTHER NAME(S): None.

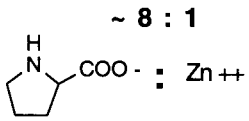
STRUCTURE: Mixture of: i) N-Acetylglucosaminyl-(Pl-4)-N-acetylmuramyl-L-alanyl-D-glutamine (GMDP), ii) Dirnethyl dioctadecylammonium. chloride (DDA), iii) Zinc L-proline salt complex (ZnPro-8) (shown below).



GMDP



DDA



Proline-Zinc (8:1)

SOURCE: i) Semisynthetic, ii) Synthetic, iii) Semisynthetic.

USES: Proprietary adjuvant formulation intended for animal and human use.

APPEARANCE: White lyophilizate.

MOLECULAR WEIGHT: i) GMDP = 695, ii) DDA = 631, iii) Pro-8:Zinc complex = ~1000

RECOMMENDED STORAGE: Store at 2-8'C.

CHEMICAL/PHYSICAL PROPERTIES: GMDP is a crystalline solid, easily dispersible in aqueous antigen solutions. The DDA by itself is very sparsely soluble (2.5 mg/L) but in the zinc L-proline complex it is easily dispersible in water. At 37C it remains in dispersion for at least 1 week. Frozen solutions can be thawed repeatedly and the dispersion of DDA remains stable. The material is soluble in 70% ethanol. The preparation is sterile and suitable for injection after reconstitution. It is contained in 5 mL glass vials with stoppers of butyl rubber. Once reconstituted, the storage must be in the frozen state to prevent microbial growth or contamination.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: All components are extensively tested for oral and parenteral toxicity and found to be nontoxic in doses well above those recommended for immunization. Zinc and L-prohne are widely used in infusions for a variety of human uses in doses larger as used in this adjuvant formula.

ADJUVANT PROPERTIES: Gerbu Adjuvant has already been tested in many applications, mainly with mice, hens, and rabbits.

- Gruhofer, N. An adjuvant based on GMDP with DDA and Zinc-L-proline complex as synergists. *E.A.G. Letters,* in press, (1994).

CONTACT(S): Dr. P. Cooper, CC Biotech Corporation, Poway, CA 92064, Ph: 619-451-9949; Fax: 619-487-8138.

COMPONENT/ADJUVANT NAME: GM-CSF

OTHER NAME(S): Granulocyte-macrophage colony stimulating factor; Sargramostim (yeast-derived rh-GM-CSF)

STRUCTURE: GM-CSF is a glycoprotein of 127 amino acids. Recombinant human GM-CSF is produced in yeast and it differs from the natural human GM-CSF by substitution of Leu for Arg at position 23.

- Walter, M. R., *et al., 1992,* Three-dimensional structure of recombinant human granulocyte-macrophage colony stimulating factor, *J. Mol. Biol. 224:1075-1085.*

Sequence of recombinant human GM-CSF (Sargramostin):

APARSPSPSTQPWEHVNAIQEALRLLNLSRDTAAEMNETVEVISEMFDLQEPTC
LQTRLELYKQGLRGSLTKLKGPLTMMASHYKQHCPPTPETSCATQIITFESFKE
NLKDFLLVIPFDCWEPVQE

SOURCE: Recombinant protein produced in yeast (*S. cerevisiae).*

USES: GM-CSF (Sargramostin) is an approved product indicated for acceleration of myeloid recovery following allogeneic and autologous bone marrow transplantation, induction and consolidation therapy for acute myeloid leukemia, for reversal of graft failure, and for mobilization of pleuipotent progenitor cells for peripherial blood cell transplantation. Reports in the literature also suggest that GM-CSF is able to activate mature granulocytes and monocyte/macrophages and dendritic cells, and may have utility as a co-adjuvant for vaccines and monclonal antibodies.

APPEARANCE: White, lyophilized powder (before reconstitution), or a clear colorless solution (after reconstitution).

MOLECULAR WEIGHT: 15,500, 16,800, and 19,500 (three bands on SDS-PAGE representing variation in glycosylation).

RECOMMENDED STORAGE: Both lyophilized GM-CSF (Sargramostin) and reconstituted product should be stored at 2-8'C. Lyophilized product ma also be frozen at -20 or -70° C.

CHEMICAL/PHYSICAL PROPERTIES: GM-CSF (Sargramostin) exists as a major species (pI 5.2) and a minor species (pI 4.5-5.2). GM-CSF (Sargramostin) shows a specific activity of 5.6×10^6 IU/mg as measured in a TF- I cell proliferation assay.

INCOMPATIBILITY: Avoid contact with proteases.

SAFETY/TOXICITY: Generally well tolerated when given for indications above. Safety data using GM-CSF as adjuvant have not been reported.

ADJUVANT PROPERTIES: This cytokine is a growth factor that stimulates non-nal myeloid precursors, and activates mature granulocytes and macrophages.

- Tao, M. H., and Levy, R., *1993,* Idiotype/granulocyte-macrophage colony stimulating factor fusion protein as a vaccine for B-cell lymphoma, *Nature 362:755-758.*

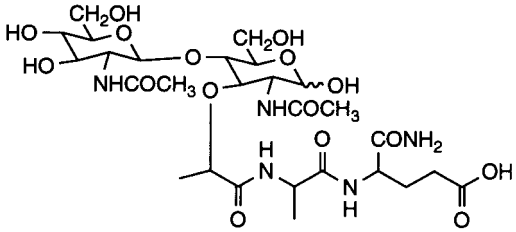
- Dranoff, *G., et al., 1993,* Vaccination with irradiated tumor cells engineered to secrete murine granulocyte macrophage colony stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity, *Proc. Natl. Acad. Sci. USA 90:3539-3543.*

CONTACT(S): Dr. Michael Widmer, Immunex Corp., Seattle, WA 98 10 1, Ph: 206-5870430; Fax: 206-587-0606.

COMPONENT/ADJUVANT NAME: GMDP

OTHER NAME(S): N-acetylglucosaminyl-(β1-4)-N-acetylmuramyl-L-alanyl-D-isoglutamine (CAS Registry Number 70280-03-4)

STRUCTURE:



SOURCE: Semi-synthetic. Disaccharide isolated from microbial origin, dipeptide wholly synthetic. US Pat. No. 4,395,399.

USES: Primary adjuvant,

APPEARANCE: White lyophilized powder.

MOLECULAR WEIGHT: 695. Contains defined low percentages of acetate and water.

RECOMMENDED STORAGE: Extremely stable at room temperature under dry conditions. For prolonged storage, desiccator at 4° C is recommended.

CHEMICAL/PHYSICAL PROPERTIES: Mp 166-170° C; pKa 5.48; Optical rotation $[\alpha]_D 20 = +2.8°$; Exists as an equilibrium mixture of two anomeric forms due to mutarotation of the -OH at C-1 of the muramic acid residue. Highly soluble in aqueous buffers, ethanol, methanol, DMF. Practically insoluble in chloroform, ether and acetonitrile.

INCOMPATIBILITY: Avoid extremes of pH.

SAFETY: Extensive Phase I systemic safety data in humans (mostly after oral administration). Single oral doses of up to 50 mg given with no side effects. Intramuscular injections of 1 mg given with minimal local reaction. LD50 in mouse = 7 gm/kg. Less pyrogenic than prototype muramyl dipeptide. In Phase II clinical trials for other applications.

ADJUVANT PROPERTIES: Highly effective primary adjuvant in a range of vehicles; aqueous buffers, mineral oil, pluronic/squalane/Tween emulsions. Also effective as oral adjuvant, enhancing mucosal IgA response.

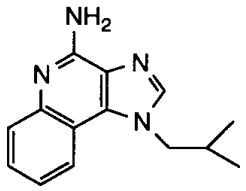
- Andronova, T. M. and Ivanov, V. T., 1991, The structure and immunomodulating function of glucosaminylmuramyl peptides. *Sov. Med. Review D. Immunology*, 4:1-63.
- Bomford, R. *et al*., 1992, The control of the antibody isotype response to recombinant human immunodeficiency antigen by adjuvants. *AIDS Res. Human Retroviruses*, 8:1765-71.
- Campbell, M. J. *et al*., 1990, Idiotype vaccination against murine B-cell lymphoma. *J. Immunol*., 145:1029-1036.

CONTACT: David Edmonds, Peptech Limited, 35-41 Waterloo Road, North Ryde, NSW 2113, Australia. Ph: 612-9870-8788; Fax: 612-9870-8786.

COMPONENT/ADJUVANT NAME: Imiquimod

OTHER NAME(S): 1-(2-methypropyl)-I*H*-imidazo[4,5-*c*]quinolin-4-amine; R-837; S26308

STRUCTURE:



SOURCE: Chemical synthesis.

- Gerster, J. F., 1987, U.S. Patent 4,680,338.
- Lagain, D., 1991, U.S. Patent 4,988,815.

USES: Included in adjuvant formulations as a primary adjuvant component.

APPEARANCE: White, fine crystalline solid.

MO**LECULAR WEIGHT:** 240.31 free base, 276.77 hydrochloride salt.

RECOMMENDED STORAGE: Solid is stable at room temperature. Shelf life is acceptable.

CHEMICALIPHYSICAL PROPERTIES: Very limited solubility as the free base. The hydrochloride salt is soluble in water at concentrations up to 10 mg/rnL. The optimal solubility for use is pH ~ 4.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: Imiquimod's safety package includes extensive evaluation with 4-month dermal and 6-month oral studies completed. In addition, no teratogenic or mutagenic effects were seen. Phase III trials as a topical antiviral agent using 5% cream, and Phase IIA trials as an oral antiviral agent using 50 and 200 mg doses of drug are ongoing.

ADJUVANT PROPERTIES: Addition of imiquimod known to induce both humoral and cell-mediated immunity via induction of cytokines from monocytes and macrophages.

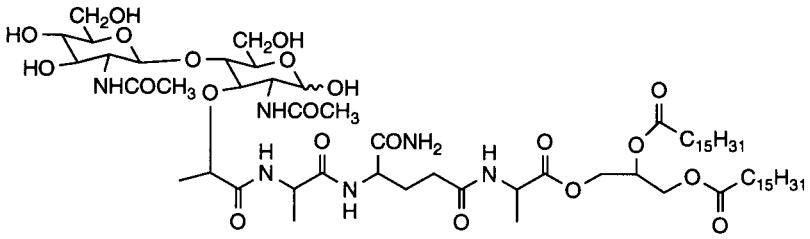
- Bernstein, D. L., *et al.,* 1993, Adjuvant effects of imiquimod on a herpes simplex virus type 2 glycoprotein vaccine in guinea pigs, J *Infect. Dis.* 167:731-735.
- Sidky, Y A., *et al.,* 1992, Inhibition of murine tumor growth by an interferon-inducing imidazoquinolinamine, *Cancer Res.* 52:3528-3533.
- Reiter, M. *J., et al.,* 1994, Cytokine induction in mice by the immunomodulator imiquimod, J. *Leukocyte Biol.* 55:234-240.

CONTACT(S): R. C. Hanson, Business Development, 3M Pharmaceuticals, St. Paul, MN 55144, Ph: 612-737-3137; Fax: 612-737-4556

COMPONENT/ADJUVANT NAME: ImmTher™

OTHER NAME(S): N-acetylglucosaminyl-N-acetyhnuramyl-L-Ala-D-isoGlu-L-Ala-glycerol dipalmitate; DTP-GDP

STRUCTURE:



SOURCE: Synthetic (US Patent 4,950,645).

USES: ImmTher™ is a potent macrophage activator, capable of inducing remission in human metastatic colorectal cancer. In-vitro and in-vivo it induces high levels of TNF, IL-1, and IL-6.The active drug compound is formulated as adjuvant in liposomes consisting of 175 mg of 1-palmitoyl-2oleoyl phosphatidy1choline and 75 mg of 1,2-dioleoyl phosphatidyl glycerol. (per 2.5 mL).

APPEARANCE: White, odorless powder. The lyophilized product is reconstituted in 2.5 mL saline to produce an initial concentration of 400 µg/mL.

MOLECULAR WEIGHT: 1316.82

RECOMMENDED STORAGE: Stable as a lyophilized powder or in solution with saline or PBS at 3-8° C for 5 years.

CHEMICAL/PHYSICAL PROPERTIES: Amphoteric molecule soluble in water, phosphate buffered saline, chloroform:methanol (7:3), and tert-butanol. The ester bond between the peptide and the lipid is subject to hydrolysis.

INCOMPATIBILITY: Avoid strong acids and bases.

SAFETY/TOXICITY: Safe in humans up to single doses of 1.2 mg/m^2 and given weekly for up to 6 months at doses of 0.8 to 1.0 mg/m^2. Major toxicity is fever, chills, and hypotension at doses of 0.8 mg/m^2 or greater. there has been no observed hernatological, hepatic or neural toxicity.

ADJUVANT PROPERTIES: The formulation is a potent macrophage activator and enhances both cellular and humoral immunity.

- Vosika, G. J. *et al*., 1991, Phase I trial of ImmTher™ a new liposome-incorporated lipophilic disaccharide tripeptide. J. Immunotherapy, 10: 256-266.
- Vosika, G. J. *et al*., 1990, Immunologic and toxicologic study of disaccharide tripeptide glycerol dipalmitoyl: A new lipophilic immunomodulator. Mol. Biother. 2: 50-56.

CONTACT: David J. Fast, Endorex Corporation, 28101 N. Ballard Drive, Lake Forest, IL 60045. Ph: 847-573-8990; Fax: 847-573-9285.

COMPONENT/ADVJVANT NAME: Immunoliposomes Containing Antibodies to Costimulatory Molecules

OTHER NAMES(S): Irnmunoliposomes prepared from Dehydration-Rehyrdation Vesicles (DRVs)

STRUCTURE: This is a DRV composed of phosphotidylcholine/cholesterol/biotinylate d-phospotidylethanolamine (PC/CH/PEB) in an molar ration of 5:5: 1. The DRVs were created from the dried film by sequentially adding a small volume of water followed by 30 seconds of vortexing. Antigen is added to the water suspension of DRV followed by repeated vortexing and lyophylization of the liposome suspension. The DRVs were hydrated with water, treated with avidin dissolved in water and washed three times in PBS prior to addition of the blotinylated-mAb.

SOURCE: Lipids are purchased from Avanti Polar Lipids, Inc., Alabaster, AL. Biotinylated mAb may be purchased from a commercial source and should not be contaminated with endotoxin.

USES: Induces delayed-type hypersensitivity, but not humoral immunity.

APPEARANCE: Cloudy suspension when in solution or white powder when dried.

MOLECULAR WEIGHT: See below for physical properties.

RECOMMENDED STORAGE: Prepare immediately before use, but may be stored at 4° C.

CHEMICAL/PHYSICAL PROPERTIES: The size of the 1iposornes has been determined to be between 1 and 8 μm by electron microscopy.

INCOMPATIBILITY: None known.

SAFETY/TOXICITY: Not known.

ADJUVANT PROPERTIES:

- Ozpolat, B., Rao, X-M., Powell, M.T., Lachman, L.B., 1998, Immunoliposomes containing antibodies to costimulatory molecules as vaccine adjuvants. *AIDS Res. and Hum. Retroviruses* 14: 409-417.
- Phillips NC, Gagne L, Tsoukas C and Dahman J., 1994, Immunoliposome targeting to murine CD4+ leucocytes is dependent on immune status. *J. Immunol*. 152:3168-3174.

CONTACT(S): Lawrence B. Lachman, Ph.D., M.B.A. Department of Cell Biology University of Texas M.D. Anderson Cancer Center Box 7101515 Holcombe Blvd., Houston, TX 77030 Ph: (713)792-8587 FAX: 713-797-9764, Email: lachman@odin.mdacc.tmc.edu

COMPONENT/ADJUVANT NAME: Interferon-γ

OTHER NAME(S): Actimmune® (rhIFN-gamma, Genentech, Inc.); immune interferon; IFN-γ; gamma-interferon

STRUCTURE: Noncovalent dimer. Low resolution crystal structure available. Monomer consists of 140 amino acids, no glycosylation or cysteines in human form. Murine form is a covalent dimer (one cysteine per monomer).

- Ealick, S. E. *et al.*, 1991, Three-dimensional structure of recombinant human interferon-γ, Science, 252:698-702.

Sequence of human interferon-gamma:

QDPYVKEAENLKKYFNAGHSDVADNGTLFLGILKNWKEESDRKIMQSQIVSFYFKLFKNFKDDQSI
QKSVETIKEDMNVKFFNSNKKKRDDFEKLTNYSVTDLNVQRKAIHELIQVMAELSPAAKTGKRKRS
QMLFRGRRASQ

SOURCE: Both human (rhIFN-gamma) and murine (rmuIFN-gamma) forms are expressed in *Escherichia coli* and distributed in a completely pure state.

USES: rhIFN-gamma (Actimmune®) is FDA-approved for use in chronic granulomastis disease (CGD). Currently, Actimmune® is in human clinical Phase III trials for renal cell carcinoma. Recombinant hIFN-gamma has been studied in humans as an adjuvant for Hepatitis B subunit antigen.

- Quiroga *et al*., 1990, *Hepatology*, 12: 661-663.

APPEARANCE: Clear aqueous solution.

MOLECULAR WEIGHT: Monomer 16.44 kD

RECOMMENDED STORAGE: 2-8° C (Do not freeze).

CHEMICAL/PHYSICAL PROPERTIES: rhIFN-gamma: pI 9.9, absorptivity = 0.75 $(mg/mL)^{-1}$ cm^{-1} at 280.4 nm, typical specific activity ~3-5 x 10^7 IU/mg. rmuIFN-gamma: absorptivity = 0.93 (mg/mL)-lcm-1 at 280 nm, typical specific activity ~0.5-1 x 10^7 IU/Mg.

INCOMPATIBILITY: Susceptible to shear-induced degradation (requires surfactants for stability), readily deamidates at high pH (> 6.5), and may be cleaved by proteases.

SAFETY/TOXICITY: rhIFN-gamma as Actimmune® is a FDA approved commercial product for human use. Standard human dose is 100 µg. High doses can cause significant side effects such as nausea, fever, and other flu-like symptoms. Effect of molecule is specific to species. Human form does not elicit toxicity in lower species at several mg/kg doses which are toxic to humans.

ADJUVANT PROPERTIES: Higher and earlier neutralizing antibody titers, increase in duration of neutralizing antibody titers, increase in MHC class 11 expression on antigen presenting cells, increase in Helper T cell levels, and an improved DTH response have all been observed when IFN-garnma was administered with an antigen. The IFN-gamma must be given at the same site and at the same time (within 6 hrs) as the antigen to have biological effect.

- Schijns, V. E. C. J. *et al.*, 1994, Modulation of antiviral immune responses by exogenous cytokines: effects of tumour necrosis factor-(x, interleukin-la, interleukin-2 and interferon-y on the immunogenicity of an inactivated rabies vaccine, *J. Gen. Virol*., 75: 55-63.
- Heath, A. W. and Playfair, J. H. L., 1992, Cytokines as immunological adjuvants, *Vaccine*, 10:427-434.
- Cao, M. *et al*., 1992, Enhancement of the protective effect of inactivated influenza virus vaccine by cytokines, *Vaccine,* 10:238-242.
- Heath, A., 1995, Cytokines as immunological adjuvants, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
- Dong, P., 1995, Cytokines as immunological adjuvants: Current status and potential applications, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Dr. Jeffrey L. Cleland, Genentech, Inc., South San Francisco, CA 94080. Ph. 415-225-3921; Fax: 415-225-3979; Email: Cleland.Jeffrey@gene.com

COMPONENT/ADJUVANT NAME: Interleukin-1β

OTHER NAME(S): IL-10; IL-1; human Interleukin 1β mature polypeptide 117-259

STRUCTURE: This protein is composed of 12 anti-parallel β-strands folded into a six-stranded barrel, with 3-fold symmetry about the axis of the barrel.

- Priestle, J. P., et al, 1988, Crystal structure of the cytokine interleukin-10. *EMB0 J.*, 7:339-43.

Sequence of IL1-β:

APVRSLNCTLRDSQQKSLVMSGPYELKALHLQGQDMEQQVVFSMSFVQGEESNDKIPVALGLKEK
NLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEINNKLEFESAQFPNWYISTSQAENMP
VFLGGTKGGQDITDFTMQFVSS

SOURCE: Recombinant mature fragment 117-259 of human interleukin-10, usually expressed in *E. coli* or other bacteria, derived from myeloid or placental libraries. Purified by sequential steps of ion exchange chromatography and gel infiltration.

USES: Primary adjuvant. Active by oral, intravenous, intraperitoneal and subcutaneous routes. It can be administered admixed with antigen or separately.

APPEARANCE: White odorless powder.

MOLECULAR WEIGHT: 17,377 kD

RECOMMENDED STORAGE: Store Lyophilized powder dry at -20° C or 4° C. The concentrated solution must be aliquoted and stored at -80° C. Avoid freeze-thawing, which results in rapid loss of activity. If diluted in solution, it tends to adhere to the vessel walls: use siliconized glass, high protein concentrations, low temperature, stabilizing proteins.

CHEMICAL/PHYSICAL PROPERTIES: The recombinant protein is quite unstable: it does not stand storage in solution at 4° C nor does it stand freeze-thawing. The protein contains unreduced cysteines which form interchain disulfide bridges upon prolonged storage (both in frozen and lyophilized conditions), with the appearance of dimeric, trimeric and multimeric molecules devoid of biological activity. pI 6.9.

INCOMPATIBILITY: Avoid proteases.

SAFETY/TOXICITY: IL-1 is a major inflammatory mediator; thus its use in vivo may have many unwanted effects. Phase I trials demonstrated severe hypotension as major side effect, as well as pain, respiratory and hermatological alterations. However, the immunostimulatory effects of IL-1 are evident at doses much lower than those yielding toxicity.

ADJUVANT PROPERTIES: It increases both T-dependent and T-independent responses to different types of antigens. Active on both primary and secondary responses.

- Staruch, M. J. and Wood, D. D., 1983, The adjuvanticity of interleukin-1 in vivo. *J. Immunol.*, 130:2191-2194.

- Nencioni, L. *et al*., 1987, In vivo immunostimulating activity of the 163-171 peptide of human IL-10. *J. Immunol*., 139:800-804.
- Frasca, D. *et al*., 1988, In vivo restoration of T cell functions by human IL-1 P or its 163-171 nonapeptide in immunodepressed mice. *J. Immunol*., 141:2651-2655 (1988).
- McCune, C. S. and Marquis, D. M., 1990, Interleukin-1 as an adjuvant for active specific immunotherapy in a murine tumor model. *Cancer Res*., 50:1212-1215.
- Heath, A., 1995, Cytokines as immunological adjuvants, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
- Dong, P., *et al*., 1995, Cytokines as vaccine adjuvants: Current status and potential applications, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Dr. Diana Boraschi, Dompè Research Center, Via Campo di Pile, 1-67100 L'Aquila, Italy, Ph: 39-862-338324; Fax: 39-862-338219.

COMPONENT/ ADJUVANT NAME: Interleukin-2

OTHER NAMES: IL-2; T-cell growth factor; aldesleukin (des-alanyl-1, serine-125 human interleukin 2); Proleukin®; Teceleukin®

STRUCTURE: Native human IL-2 contains 133 amino acids (see below); aldesleukin contains 132 amino acids. IL-2 exists as six alpha helical domains, termed A to F. Glycosylation not essential for function.

• Rosenberg, S. A. *et al*., 1983, Biological activity of recombinant human interleukin-2 produced in *Escherichia coli, Science,* 223: 1412-14.
• Brandhuber, B. J. *et al*., 1987, Three dimensional structure of interleukin-2, *Science*, 238: 1707-09.
• Ju, G. *et al*., 1987, Structure function analysis of human interleukin-2: Identification of amino acid residues required for biological activity. *J. Biol. Chem*., 262: 5723-31.

Sequence of human IL-2:

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLE
EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

SOURCE: Recombinant protein expressed in *E. coli.*

USES: As a primary adjuvant, co-emulsified with antigens and lipids, with polyethylene glycol modified long acting form (PEG IL-2), or liposome encapsulated sustained release dosage form. Aldesleukin (Proleukin@) is a FDA-licensed agent for treatment of metastatic renal cell carcinoma.

APPEARANCE: Lyophilized, white to off-white colored solid, Reconstituted with water for injection to give a clear, colorless solution.

MOLECULAR WEIGHT: 15.3 kD.

RECOMMENDED STORAGE: Store lyophilized aldesleukin solid at 2-8° C. Store the reconstituted product at 2-8° C for no longer than 48 hours. Reconstituted solution diluted with 5% dextrose to a 200 µg/mL IL-2 concentration stable in plastic syringes at 2-8° C for 14 days. Store lyophilized PEG IL-2 at -20° C and reconstituted PEG IL-2 at 2-8° C for up to 28 days. Storage stability of liposomal dosa form unknown.

CHEMICAL/ PHYSICAL PROPERTIES: Relatively hydrophobic protein with moderate aqueous solubility (-1 mg/mL). Major pl = 8.0. Adsorbs to glass and plastic surfaces below concentrations of 10 ~µg/mL or less, this can be prevented by having 0.1% human albumin present in the diluting solution prior to adding aldesleukin. Potential degradation pathways: methionine oxidation, aggregation, dimer and higher oligomer formation and deamidation.

INCOMPATIBILITY: Aldesleukin is not compatible with sodium chloride for injection, solutions with high ionic strength or containing preservatives. May degrade with proteases; avoid solutions of low or high pH extremes. Compatible with mineral oil and other lipoidal adjuvants (e.g., DDA, Avridine®).

SAFETY/TOXICITY: Frequency and severity of adverse reactions are generally dose-related. The most frequently reported serious adverse reactions include hypotension, renal dysfunction, dyspnea and mental-state

changes. Further descriptions are indicated in the Proleukin& (Aldesleukin for Injection) package insert (Chiron Corporation, Emeryville, CA, U.S.A.).

- Rosenberg, S. A. *et al*., 1985, Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *New Eng. J. Med*., 313:1485-92.
- Kroemer, G. *et al*., 1992, Interleukin-2, a pro-autoinimune lymphokine that interferes with post-deletional tolerance. *Sem. Immunol*., 4:167-179.

ADJUVANT PROPERTIES: IL-2 supports the growth and proliferation of antigen-activated T lymphocytes and plays a central role in the cascade of cellular events involved in the immune response. Proliferating T-cells also produce a variety of other lymphokines which may modulate other arms of the immune system. In view of these direct and indirect actions of IL-2 on the immune response, IL-2 may function as an adjuvant to vaccination by increasing the specific and durable response to vaccine immunogens. Low doses may give up to 25-fold increase in adjuvant effect, with inhibition of adjuvant effect at high doses. May induce cellular immunity when given systemically, and IgA when administered at a mucosal surface.

- Weinberg, A. and Merrigan, T. C., 1988, Recombinant interleukin-2 as an adjuvant for vaccine-induced protection, *J. Immunol*., 140: 294-299.
- Nunberg, J. H. *et al*., 1989, Interleukin 2 acts as an adjuvant to increase the potency of inactivated rabies virus vaccine, *Proc. Nat'l Acad. Sci*., 86: 4240-43.
- Ho, R. J. Y. *et al*., 1991, A potentially useful vaccine adjuvant., In: Topics in Vaccine Ad-juvant Research D. R. Spriggs and W. C. Koff, Eds. CRC Press, Boca Raton, p 69-76.
- Ho, R. J. *et al*., 1992, Liposome-formulated interleukin-2 as an adjuvant for the treatment of recurrent genital HSV-2 in guinea pigs with recombinant HSV glycoprotein gD. *Vaccine*, 10:209-13.
- Hughes, H. P. A. *et al*., 1991, Immunopotentiation of bovine herpes virus subunit vaccination by IL-2. *Immunol*., 74:461-466.
- Hughes, H. P. A. *et al*., 1992, Multiple administration of with cytokines potentiates antigen specific responses to subunit vaccination with bovine herpes virus-1 glycoprotein IV. *Vaccine*, 10: 226-30 (1992).
- Tan, L. and Gregoriadis, G., 1989, Effect of interleukin-2 on the immunoadjuvant action of liposomes. *Biochem. Soc. Trans*, 17:693-694.
- Mbwuike, I. N. *et al*., 1990, Enhancement of the protective efficacy of the inactivated influenza A virus vaccine in aged mice by IL-2 liposomes. *Vaccine*, 8: 347-352.
- Heath, A., 1995, Cytokines as immunological adjuvants, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
- Ho, R. J. Y. *et al*., 1995, Cytokines as vaccine adjuvants: Current status and potential applications, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT: Huw Hughes, M6 Pharmaceuticals, Inc., Yonkers, NY 10701. Ph: 914-476-6799; Email 74577.345@compuserve.com; Professional Services, Chiron Therapeutics, A Division of Chiron Corporation, Emeryville, CA, U.S.A.

COMPONENT/ADJUVANT NAME: Interleukin-7

OTHER NAME(S): IL-7

STRUCTURE:
- Goodwin, R. G. *et al*., 1989, Molecular cloning and growth factor activity on human and murine B-lineage cells. *Proc. Natl. Acad. Sci*. USA, 86:302-306.

Sequence of IL-7:

MFHVSFRYIFGLPPLILVLLPVASSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFK
RHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTYSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

SOURCE: Recombinant protein expressed *E. coli*, Immunex Corp., Sterling Winthrop Pharmaceuticals.

USES: Primary adjuvant, liposome formulated sustained release form. Co-emulsified with antigen and lipids.

APPEARANCE: Clear aqueous solution.

MOLECULAR WEIGHT: 25 kD

RECOMMENDED STORAGE: 4º C for both IL-7 and liposome-formulated IL-7.

CHEMICAL/PHYSICAL PROPERTIES: Reasonable solubility in water (-1 mg/mL).

INCOMPATIBILITY: Avoid proteases.

SAFETY/TOXICITY: Unknown.

ADJUVANT PROPERTIES:
- Bui, T., *et al*., 1994, Biologic response of recombinant interleukin-7 on herpes simplex virus infection in guinea pigs. *Vaccine*, 12:646-652.
- Bui, T., *et al*., 1994, Effect of MTP-PE liposomes and IL-7 on induction of antibody and cell-mediated immune responses to a recombinant HIV envelope protein. *J. AIDS*., in press.
- Heath, A., 1995, Cytokines as immunological adjuvants, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
- Dong, P., *et al*., 1995, Cytokines as vaccine adjuvants: Current status and potential applications, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Dr. Rodney Ho, University of Washington, Seattle, WA Ph: 206-685-3914; Fax: 206-543-3204.

COMPONENT/ADJUVANT NAME: Interleukin-12

OTHER NAME(S): IL-12; natural killer cell stimulatory factor (NKSF); cytotoxic lymphocyte maturation factor (CLMF)

STRUCTURE: IL-12 is a heterodimeric protein composed of two disulfide-bonded glycoprotein subunits approximately 35 and 40 kDa in size. The two subunits represent two separate, unrelated gene products that have to be coexpressed to yield the secreted, bioactive, heterodimeric lymphokine.

- Gubler, U., *et al.*, 1991, Coexpression of two distinct genes is required to generate secreted, bioactive cytotoxic lymphocyte maturation factor, *Proc. Natl. Acad. Sci. USA* 88:4143-4147.
- Wolf, S. R, *et al.*, 1991, Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells, J. Immunol. *146:3074-3081*.
- Schoenhaut, D. *S.*, *et al.*, 1992, Cloning and expression of murine IL-12, J. Immunol. *148:3433-3440.*

Sequence of 40-kDa subunit of human IL- 12:

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKT
LTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKT
FLRCEAKNYSGRFrCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVR
GDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKP
DPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKD
RVFrDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS

Sequence of 35-kDa subunit of human IL-12:

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITK
DKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDL
KMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSL
EEPDFYKTKIKLCILLHAFRIRAVTIDRVTSYLNAS

SOURCE: Recombinant protein purified from the medium of cultures of CHO cells transfected with IL-12 cDNAs. Natural sources of the protein include activated monocyte/macrophages and B lymphocytes.

USES: Included as a primary adjuvant component to enhance Th1-dependent cell-mediated immunity.

MOLECULAR WEIGHT: Protein: 57,200; glycosylated protein: ~70,000.

RECOMMENDED STORAGE: Store IL-12 at -70º C in pH 7 buffer free of calcium, magnesium, and potassium salts (1 mg/mL). Maximum two freeze-thaws pending further investigation. Storage in opaque polypropylene containers is preferable.

CHEMICAL/PHYSICAL PROPERTIES: Three major bands in the pI range of 4.5 to 5.3. IL-12 is most stable at pH 7 at 10 µg/mL and greater. At lower and higher pH, significant loss occurs via either protein breakdown or protein adsorption to glass. Stress conditions such as heating and shaking promote aggregation and protein loss.

INCOMPATIBILITY: Avoid proteases.

SAFETY/TOXICITY: Clinical trials are in progress in AIDS and oncology (GI and Roche). In mice and primates repetitive daily dosing with $\geq$ 50 µg/kg may result in anemia, leukopenia, hepatotoxicity, skeletal muscle necrosis (seen only in mice), and vascular leak. It has also been shown in some species that repetitive daily dosing of >1 pg/kg results in the same side effects.

ADJUVANT PROPERTIES: Enhances Th1-dependent cell-mediated immune responses including cytolytic T-lymphocyte responses. Suppresses Th2-dependent humoral immune responses such as IgE responses but may enhance production of Ig isotypes, such as IgG2a in mice, associated with Thl responses.

- Afonso, L. C. C., *et al*., 1994, The adjuvant effect of interleukin-12 in a vaccine against *Leishmania major*. *Science* 263: 235-237.
- Gately, M. K., *et al*., 1994, Administration of recombinant IL-12 to normal mice enhances cytolytic lymphocyte activity and induces the production of IFN-g in vivo. *Intl. Immunol*. 6:157-167.
- McKnight, A.J. *et al*., 1994, Effects of IL-12 on helper T cell-dependent immune responses in vivo. *J. Immunol*., 152: 2172-2179.
- Heath, A., 1995, Cytokines as immunological adjuvants, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
- Dong, P., *et al*., 1995, Cytokines as vaccine adjuvants: Current status and potential applications, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACTS: Maury K. Gately or Alvin S. Stem, Hoffmann-La Roche Inc., Nutley, NJ 07110-1199. Ph: 201-235-5720; Fax: 201-235-5279. Also: Stan Wolf, Genetics Institute, Cambridge, MA Ph: 617-498-8134; Fax: 617-876-1504.

COMPONENT/ADJUVANT NAME: ISCOM(s)™

OTHER NAME(S): Immune stimulating complexes

STRUCTURE: ISCOMs are a complex composed of typically 0.5% Quillaja saponins, 0.1% cholesterol, 0.1% phospholipid, and antigen in phosphate-buffered saline (PBS). Occasionally, surfactants are used t are ISCOMs (such as Mega 10) but are removed from the final formulation before use.

SOURCE: The adjuvant-active components of ISCOMs are derived by aqueous extraction of the bark of *Quillaja saponaria* and are further purified by chromatography. Quil A is a purified form of this. Further chromatographic purification provides components with high adjuvant activity and ISCOM-forming properties (see Iscoprep 7.0.3™).

USES: ISCOMs are powerful immunomodulators. Steric presentation of epitopes, and CTL responses are maximized by the incorporation of immunogens into ISCOMs. Iscotec holds patents covering the use of ISCOMs.

APPEARANCE: ISCOMs form a clear product in solution.

MOLECULAR WEIGHT: A selection of components with various molecular weights: cholesterol, 386.7; Quil A ~2000; DMPC, 677.9.

RECOMMENDED STORAGE: Store ISCOMs at conditions compatible with the incorporated antigen(s). In general, storage in physiological buffers 4-8º C or may be stored at -70º C.

CHEMICAL/PHYSICAL PROPERTIES: ISCOMs are stable complexes which show good suspendability (>100 mg/mL) in buffer.

INCOMPATIBILITY: Avoid exposure to alkaline pH > 8.0.

SAFETY/TOXICITY: Studies are under progress, and to date have shown no adverse effects in several animal species. ISCOMs have not shown hemolytic activity at normally-administered dose levels.

ADJUVANT PROPERTIES: The ISCOM is an antigen-presenting structure and has been studied for a number of antigens. ISCOMs generate long-lasting biologically functional antibody response, even in the presence of maternal antibodies. Protective immunity and a functional cell-mediated immune response, including Class I restricted CTLs have been reported in several systems. ISCOMs have generally been administered subcutaneously or intramuscularly but non-parenteral administrations (intranasal and oral) have also proven to be effective.

- Morein, B. *et al*., 1994, Iscom, a novel structure for antigenic presentation of membrane proteins from enveloped viruses. *Nature*, 308:457.

- Classen, I. Osterhaus, A., 1992, The iscom structure as an immune enhancing moiety: Experiences in viral systems. *Res. Immunol.*, 143:531-541.
- Hoglund, S. *et al*., 1989, Iscoms and immunostimulation with viral antigens. In: *Subcell. Biochem.* Harris, Ed., 39, Plenum Publishing, New York.
- Osterhaus, A. and Rimmelzwan, G. F., 1995, A novel generation of viral vaccines based on the ISCOM matrix, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): ISCOTEC AB, Box 7418, S-10391 Stockhohn, Sweden Ph: 46-8-6797810; Fax: 46-18674376. Also: Bror Morein, National Veterinary Institute, Uppsala, Sweden Ph: 46-18-174571; Fax: 4618-504-603.

COMPONENT/ADJUVANT NAME: Iscoprep 7.0.3.™

OTHER NAME(S): None

STRUCTURE: Complex of saponin derivatives.

SOURCE: Purified by aqueous extraction of the bark of Quillaja saponaria and are further purified by chromatography to produce Iscoprep 7.0.3™, a carefully selected mixture of saponin components with adjuvant activity and ISCOM-forming capacity.

USES: Iscoprep 7.0.3 ™ is used to produce ISCOMs.

APPEARANCE: Iscoprep 7.0.3™ is a light tan to white lyophilized powder.

MOLECULAR WEIGHT: Iscoprep 7.0.3™ is a selection of components with various molecular weights around 1800 to 2200.

RECOMMENDED STORAGE: Store powder at 4-8º C in the dark, solutions at -20º C.

CHEMICAL/PHYSICAL PROPERTIES: The mixture of saponin components in Iscoprep 7.0.3™ binds to cholesterol and phospholipid to form ISCOMs. ISCOMs are stable complexes made up of amphiphilic molecules.

INCOMPATIBILITY: Avoid exposure to alkaline pH >8. Studies are under progress. Iscoprep 7.0.3 ™ does not show hemolytic activity in ISCOMs at normally-administered dose levels.

ADJUVANT PROPERTIES: Iscoprep 7.0.3™ is used to formulate ISCOMs. The ISCOM as an antigen-presenting structure and has been studied for a number of antigens. ISCOMs generate long-lasting biologically functional antibody response, even in the presence of maternal antibodies. Protective immunity and a functional cell-mediated immune response, including Class I restricted CTLs have been reported in several systems. ISCOMs have generally been administered subcutaneously or intramuscularly but non-parenteral administrations (intranasal and oral) have also proven to be effective.

- Morein, B. *et al*., 1984, Iscom a novel structure for antigenic presentation of membrane proteins ft= enveloped viruses. *Nature*, 308:457.
- Classen, I. Osterhaus, A., 1992, The iscom structure as an immune enhancing moiety: Experiences in viral systems. *Res. Immunol*., 143:531-541.
- Hoglund, S. *et al*., 1989, Iscoms and immunostimulation with viral antigens. In: *Subcell. Biochem.* Harris, Ed., 39, Plenum Publishing, New York.
- Osterhaus, A. and Rimmelzwan, G. F., 1995, A novel generation of viral vaccines based on the ISCOM matrix, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): ISCOTEC AB, Box 7418, S-10391 Stockholm, Sweden Ph: 46-8-6797810; Fax: 46-18674376.

COMPONENT/ADJUVANT NAME: Liposomes

OTHER NAME(S): Liposomes (L) containing protein or Th-cell and/or B-cell peptides, or microbes with or without co-entrapped interieukin-2, BisHOP or DOTMA (see below). A, [L (Antigen)]; B, [L (IL-2 or DOTMA or BisHOP + Antigen)]; C, [L (Antigen)-mannose]; D, [L (Th-cell and B-cell epitopes)]; E, [L (microbes)].

STRUCTURE(S): A: Multilamellar liposornes prepared by the dehydration-rehydration method (average diameter 600-800 nm) composed of egg phosphatidy1choline (PC) or distearoyl phosphatidylcholine (DSPQ and equimolar cholesterol and containing antigens such as tetanus toxoid and synthetic Th-cell peptides. 13: As in A with IL-2 (10^3–10^4 Cetus units) co-entrapped with the antigen in the aqueous phase or with 1,2-bis (hexadecylcycloxy)-3-trimethylaminopropane-HCL (BisHOP) or *N-(2,3*-dioleyloxy*)-NNN*-triethylammonium (DOTMA) incorporated into the lipid phase of liposomes (0.8:1.0:0.2 molar ratio for PC or DSPC, cholesterol and DOTMA or BisHOP). C, as in A with marmosylated albumin covalently coupled to the surface of antigen-containing liposomes. D: As in A with Th-cell and B-cell peptides co-entrapped in the aqueous phase. E: Giant liposornes (average diameter 5-9 µm) prepared as in A or by a solvent-spherule evaporation method, composed of PC or DSPC, cholesterol, triolein (TO), and phosphatidylglycerol (PG) (4:4:1:2 molar ratio) and containing killed or live *Bacillus subtilis* or killed Bacille Calmette-Guérin (BCG) with or without co-entrapped tetanus toxoid.

SOURCE: PC, DSPC, and PG in pure forin from Lipid Products, Nuthill, Surrey, U.K.; TO in pure form from Sigma Chemical Co., Poole, Dorset, U.K.; recombinant interieukin-2 (des-Ala_1-Ser_{125} mutein; 3 x 10^6 Cetus units/mg) obtained from Cetus Corporation, Emeryville, CA; BisHOP and DOTMA obtained from Syntex Research, Palo Alto, CA.

APPEARANCE: White, opalescent colloidal suspensions (A-E).

MOLECULAR WEIGHT: Equal to the sum of the molecular weights of the components used in each of the formulations. The molecular weight of antigen will vary according to its type.

RECOMMENDED STORAGE: Store formulations at 4º C when in liquid form. Freeze dried formulations stored at 4 or -20º C. Liquid formulations stable (in terms of entrapped antigen release) for at least 1 year when sterile. Precipitated liposomes made into suspended by light vortexing.

CHEMICAL/PHYSICAL PROPERTIES: Liposomes are stable at a pH range of 1-10; however, neutral pH is recommended when cytokines and certain antigens are present. Lipid components of liposomes are soluble in chloroforrn and are stable for at least 1 year at -20º C.

INCOMPATIBILITY: Formulations unstable in the presence of detergents (e.g., Triton X-100).

SAFETY/TOXICITY: Liposomes as such composed of PC and cholesterol have been administered to humans in numerous clinical trials with no adverse effects. None of the formulations (A-E) have been tested in humans.

IMMUNOLOGICAL ADJUVANT AND VACCINE CARRIER PROPERTIES: A, potentiation of immune responses (IgGl, IgG2a, IgG2b, or IgG3) to protein and peptide antigens; choice of phospholipid depends on antigen; a high mass ratio of phospholipid to antigen (e.g., 10^3) optimizes immune responses. B, IL-2, DOTMA, and BisHOP potentiate immune responses to antigens further, acting as co-adjuvants. C, targets liposornes to macrophages with immune responses being greater than with conventional liposomes. D, liposomes act as carrier of Th-cell peptide antigen which provides help for co-entrapped B-cell antigen to overcome genetic restriction

and induce immunological memory. E, liposomes may act as carriers of attenuated or live microbial vaccines to deliver microbes and co-entrapped soluble antigens or cytokines simultaneously to antigen-presenting cells or to protect entrapped vaccines from interaction with maternal antibodies or antibodies to vaccine impurities in preimmunized subjects.

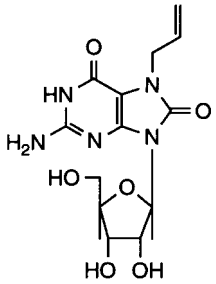
- Gregoriadis, G., 1990, Immunological adjuvants: A role for liposomes, *Immunol. Today* 11:89-97.
- Davis, D., and Gregoriadis, G., 1987, Liposomes as adjuvants with immunopurified tetanus toxoid: Influence of liposomal characteristics, *Immunology* 61:229-234.
- Gregoriadis, G., *et al*., 1987, Liposomes as immunological adjuvants: Antigen incorporation studies, *Vaccine* 5: 143-149.
- Tan, L., and Gregoriadis, G., 1989, The effect of interleukin-2 on the immunoadjuvant action of liposomes, *Biochem. Soc. Trans.* 17:693-694.
- Garcon, *N., et al*., 1988, Targeted immunoadjuvant action of tetanus toxoid-containing liposomes coated with mannosylated albumin, *Immunology* 64:743-745.
- Kahl, K. L., *et al*., 1989, Vaccination against murine cutaneous leishmaniasis using L. Major antigen/liposomes: Optirntization and assessment of the requirement for intravenous immunization, J. *Immunol.* 142:4441-4449.
- Antimisiaris, S., *et al*., 1993, Liposomes as vaccine carriers: Incorporation of soluble and particulate antigens in giant vesicles, J. *Immunol. Methods* 166:271-280.
- Gregoriadis, G., *et al*., 1993, Liposome-entrapped T-cell peptide provides help for co-entrapped B-cell peptide to overcome genetic restriction in mice and induce immunological memory, *Immunology* 80:535-540.

CONTACT(S): Professor Gregory Gregoriadis, Centre for Drug Delivery Research, School of Pharmacy, University of London, 29-39 Brunswick Square, London WCIN 1AX, U.K., Ph:, +44-171-7535822; Phone/Fax +44-171-7535820.

COMPONENT/ADJUVANT NAME: Loxoribine

OTHER NAME(S): 7-allyl-8-oxoguanosine

STRUCTURE:



SOURCE: Synthetic (US Patent 5,011,828).

USES: Primary adjuvant for antibody responses to a wide variety of antigen types in a variety of species. Typical dose in mice: 1-3 mg/25 g mouse. In humans: 10 mg/kg has been used safely. Optimal dose unknown.

APPEARANCE: White, odorless crystalline powder.

MOLECULAR WEIGHT: 339

RECOMMENDED STORAGE: Store solid under low humidity at -20º C. Stable in solution for at least 4 weeks; found to be very stable at pH 8-11.

CHEMICAL/PHYSICAL PROPERTIES: Hydrophobic, lipophilic molecule. Soluble in DMSO, DMF, and aqueous media at alkaline pH. Mp=234º C. pKa=8.92.

INCOMPATIBILITY: Precipitates at low H

SAFETY/TOXICITY: Phase I clinical trial complete, without toxicity greater than grade I. No reported toxicity in a lower dose, phase I/II clinical trial. Main side effects noted resemble those of interferon and are transient.

ADJUVANT PROPERTIES: Augmentation of CTL-mediated, NK cell-mediated, macrophage mediated, and LAK cell-mediated cytotoxicity. Inducer of cytokines: IFN(α/β/γ, TNFα, TNFβ, IL-1α, IL-6. Up regulates humoral immune responses in immunodeficiency. Acts as a surrogate Th signal.

- Goodman, M. G. and Weigle, W. 0., 1985, Enhancement of the human antibody response by C8substituted guanine ribonucleosides in synergy with interleukin 2. *J. Immunol.*, 135:3284 -3288.
- Feldbush, T. L. and Ballas, Z. K., 1985, Lymphokine-like activity of 8-mercaptoguanosine: induction of T and B cell differentiation. *J. Immunol.*, 134:3204-3211.
- Goodman, M. G. *et al*., 1991, C-kinase independent restoration of specific irnmune responsiveness in common variable immunodeficiency. *Clin. Immunol. and Immunopath.*, 59:26-36.
- Goodman, M. G. and Weigle, W. 0.,1983, T cell-replacing activity of C8-derivatized guanine ribonucleosides. *J. Immunol.*, 130:2042-2045.

- Pope, B. L. *et al*., 1993, Loxoribine (7-allyl-8-oxoguanosine) activates natural killer cells and primes cytolytic precursor cells for activation by IL-2. *J. Immunol.*, 151:3007-3017.
- Goodman, M., 1995, A new approach to adjuvants: Immunopotentiation by intracellular T helper-like signals delivered by loxoribine, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Dr. Michael G. Goodman, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037, Ph: 619-554-8131; Fax: 619-554-6705.

COMPONENT/ADJUVANT NAME: LT-OA or LT Oral Adjuvant

OTHER NAME(S): *E. coli* labile enterotoxin protoxin

STRUCTURE: Polypeptide consisting of one 28 kD A subunit (toxic component consisting of the A, chain of 21 kD and the A2 chain of 7 kD) and five 11.6 kD B subunits (binding component).

- Sixma, T. K. *et al*., 1991, Crystal structure of a cholera toxin-related heat-labile enterotoxin from *E. coli.* Nature 351: 371-377.

Sequence of *E. coli* heat labile enterotoxin subunit A:
NGDKLYRADSRPPDEIKRSGGLMPRGHNEYFDRGTQMNINLYDHARGTQTGFVRYDDGYVSTSLS
LRSAHLAGQSILSGYSTYYIYVIATAPNMFNVNDVLGVYSPHPYEQEVSALGGIPYSQIYGWYRVNF
GVIDERLHRNREYRDRYYRNLNIAPAEDGYRLAGFPPDHQAWREEPWIHHAPQGCGDSSRTITGD
TCNEETQNLSTIYLRKYQSKVKRQIFSDYQSEVDIYNRIRNEL*

Sequence of *E. coli* heat labile enterotoxin subunit B:
APQSITELCSEYRNTQIYTINDKILSYTESMAGKREMVIITFKSGATFQVEVPGSQHIDSQKKAIERMK
DTLRITYLTETKIDKLCVWNNKTPNSIAAISMEN*

SOURCE: Toxigenic *Escherichia coli,* either partially purified or recombinant, extracted under conditions that inhibit proteolysis and thus inhibit conversion to active toxin. Commercially available from Berna Products Corp., Coral Gables, FL.

USES: Sole active component of orally-administered adjuvant.

APPEARANCE: White, odorless powder.

MOLECULAR WEIGHT: 84,000 to 94,000 depending on the assay method.

RECOMMENDED STORAGE: Store lyophilized solid at -20º C. Solutions of LT-OA at 1 mg/mL at pH 7.5 in non-phosphate buffers may be stored at 5º C for 1-2 years.

CHEMICAL/PHYSICAL PROPERTIES: Amphiphilic molecule with low solubility in water at neural pH. pI 8.0. Not stable in phosphate buffers. Oxidizes on long-term storage to form intramolecular disulfide bonds.

INCOMPATIBILITY: Avoid proteases. Incompatible with phosphate buffer.

SAFETY/TOXICITY: Non-toxic at adjuvant active doses in mouse, rabbit and monkey. Human Phase I clinical trials scheduled to begin July, 1994.

- J.A. Majde, 0. Pavlovskis, S. Baqar, J.M. Katz, R.I. Walker and J.D. Clements, 1994, *Escherichia coli* heat-labile enterotoxin, an oral adjuvant for protection against mucosal pathogens. In Adjuvants-Theory and Practical Applications D. Stewart-Tull, Ed., John Wiley, London.

ADJUVANT PROPERTIES: For inducing mucosal and systemic immunity (both humoral [including IgA and IgG2, isotypes] and cell-mediated) to killed microorganisms or peptide antigens mixed with it in neutral non-phosphate buffered saline, with/without sodium bicarbonate.

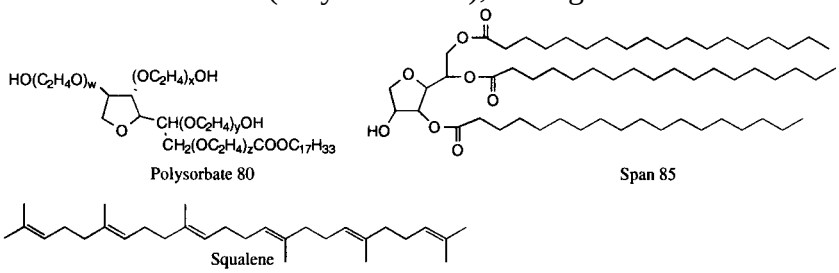
- Clements, J. D. *et al*., 1988, Adjuvant activity of *Escherichia coli* heat-labile enterotoxin and effect on the induction of oral tolerance in mice to unrelated protein antigens. *Vaccine*, 6: 269-277.
- Walker, R. I. and Clements, J. D., 1993, Use of the heat labile toxin of entertoxigenic *Escherichia coli* to facilitate mucosal immunization. *Vaccine Res*., 2:1-10.
- Rollwagen, F. M. *et al*., 1993, Killed *Campylobacter* elicits immune response and protection when administered with an oral adjuvant. *Vaccine* 11:1316-1319.
- Baqar, S. *et al*., 1995, Safety and immunogenicity of a prototype oral whole cell killed *Campylobacter* vaccine administered with a mucosal adjuvant in non-human primates. *Vaccine* 3: 22-28.

CONTACT(S): Dr. Jeannine A. Majde, Program Manager, Biomedical Science and Technology, Office of Naval Research, Arlington, VA 22217-5660, Ph: 703-696-4055; Fax: 703-696-1212; E-mail: majdej@onrhq.onr.navy.mil

COMPONENT/ADJUVANT NAME: MF59

OTHER NAME(S): None

STRUCTURE: Squalene/water emulsion. Composition: 43 mg/mL squalene, 2.5 mg/mL polyoxyethylene sorbitan monooleate (Polysorbate 80), 2.4 mg/mL sorbitan trioleate (Span 85).



SOURCE: Chiron Corporation, Emeryville, CA.

USES: Intramuscular adjuvant.

APPEARANCE: White liquid

MOLECULAR WEIGHT: n/a

RECOMMENDED STORAGE: 2-8° C, inert gas overlay.

CHEMICAL/PHYSICAL PROPERTIES: Low viscosity aqueous emulsion, biodegradable. Particle size 200-300 nm.

INCOMPATIBILITY: Unstable upon freezing. Exposure to pH extremes results in hydrolysis of detergent components. Components are susceptible to oxidation in presence of 0_2, peroxide, metals.

SAFETY/TOXICITY: Minor reactogenicity upon intramuscular injection of humans in combination with HSV or HIV antigens.

ADJUVANT PROPERTIES: Intramuscular injection in combination with a variety of subunit antigens results in elevated humoral response, increase T cell proliferation and presence of cytotoxic lymphocytes.

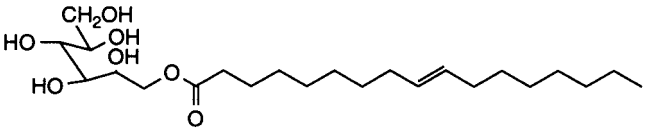
- Sanchez-Pestador, L., *et al*., 1988, The effect of adjuvants on the efficacy of a recombinant herpes simplex glycoprotein vaccine, *J. Immunol*., 141:1720-1727.
- Van Nest, G. A., *et al*, 1992, Advanced adjuvant formulations for use with recombinant subunit vaccines in: Vaccines 92, Ed by F. Brown, RM. Chanock, H. S. Ginsberg and R. A. Lerner, Cold Spring Harbor Press, Plainview, NY, pg 57.
- Van Nest, G. A. *et al*., 1995, MF59: Design and evaluation of a safe and potent adjuvant for human vaccines, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Dr. Gary Ott, Chiron Corporation, Ph: 510-923-2964; Fax: 510-601-2586; Gail Barchfeld, Chiron Corporation, Ph. 510-923-4265; Fax: 510-601-2586.

COMPONENT/ADJUVANT NAME: MONTANIDE ISA 51

OTHER NAME(S): Purified IFA; Incomplete Freund's adjuvant.

STRUCTURE: Mannide oleate (mostly mannide monooleate, esters of mannitol and oleic acids - an example shown below) (MONTANIDE 80) in mineral oil solution (DRAKEOL 6VR).



SOURCE: Manufactured by SEPPIC.

USES: "Ready to use" oil for water-in-oil emulsion adjuvants. For human use. Final injection product usually 50% Montanide ISA 51 (0.5-1 mL injection volume).

APPEARANCE: Limpid clear yellow liquid.

RECOMMENDED STORAGE: Store at 4° C or room temperature under nitrogen. Stable at room temperature for at least 1 year. Store at physiological pH.

CHEMICAL/PHYSICAL PROPERTIES: Acid value: 0.5 maximum; saponification value: between 16 and 20; hydroxyl value: between 9 and 13; peroxide value: 2 maximum; iodine value: between 5 and 9; water content: 0.5 maximum; refractive index at 25° C: between 1.455 and 1.465; density (at 20° C): about 0.85; viscosity (at 20° C): about 50 mPas. Water insoluble. DRAKEOL 6VR is a special pharmaceutical grade mineral oil that contains paraffin oil with linear and ramified hydrocarbons in the range C_{14}-C_{26} (mean C_{24}).

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: LD50 (mice) by i.p. route, > 22g/kg. LD50 (rats) by oral route, > 2 g/kg, Non irritating (skin) in rabbits, Slight irritancy (ocular) in rabbits. No abnormal toxicity in mice or guinea pigs. Acute toxicity by i.m. injection (rats), > 5g/kg. Pyrogen-free. Ames and Mouse Micronucleus test (Montanide 80) - no effect.

ADJUVANT PROPERTIES: Addition of MONTANIDE ISA 51 induces humoral and cell-mediated immunity with various antigens.

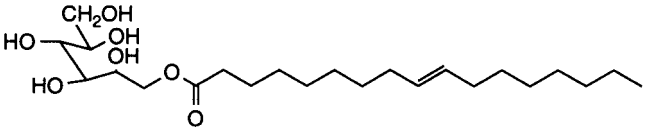
- Audibert, F. M. and Lise., L. D., 1993, Adjuvant: Current status, clinical perspectives and future prospects. *Immunology Today*, 14:281-284.
- Ganne, V. *et al*., 1994, Enhancement of the efficacy of a replication-defective adenovirus-vectored vaccine by the addition of oil adjuvants. *Vaccine*,12:1190-1196.

CONTACT(S): SEPPIC, 75321 Paris Cedex 07, France, Ph: 33140 62 57 30; Fax: 33140 62 52 53.

COMPONENT/ADJUVANT NAME: MONTANIDE ISA 720

OTHER NAME(S): metabolizable oil adjuvant

STRUCTURE: A highly refined emulsifier from the mannide monooleate family (an example of mannide monooleate shown below) in a natural metabolizable oil solution. The exact nature of the emulsifier and the metabolizable in MONTANIDE ISA 720 is proprietary, but can be disclosed under specific agreement with SEPPIC.



SOURCE: manufactured by SEPPIC.

USES: "Ready to use" oil for water-in-oil emulsion adjuvants. Final injection product usually 70% Montanide ISA 720 (0.5-1 mL injection volume).

APPEARANCE: Yellow, odorless liquid

RECOMMENDED STORAGE: Store at 4° C or room temperature under nitrogen. Stable at room temperature for at least 1 year. Store at physiological pH.

CHEMICAL/ PHYSICAL PROPERTIES: Acid value 0.5 maximum; saponification value: between 17 and 21; hydroxyl value: between 9 and 12; peroxide value: 5 maximum; iodine value: between 320 and 350; water content: 0.5 maximum; refractive index (at 25° C) about 1.492; density (at 20° C) about 0.86; viscosity (at 20° C) : about 15 mPas. Water insoluble. DRAKEOL 6VR is a special pharmaceutical grade mineral oil that contains paraffin oil with linear and ramified hydrocarbons in the range C_{14}-C_{26} (mean C_{24}).

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: LD50 (mice) by i.p. route, > 22g/kg. LD50 (rats) by oral route, > 2 g/kg, Non irritating (skin) in rabbits, Slight irritancy (ocular) in rabbits. No abnormal toxicity in mice or guinea pigs. Acute toxicity by i.m. injection (rats), > 5g/kg. Pyrogen-free. Ames and Mouse Micronucleus test (Montanide 80) - no effect.

ADJUVANT PROPERTIES: Addition of MONTANIDE ISA 720 induces humoral and cell-mediated immunity with various antigens.

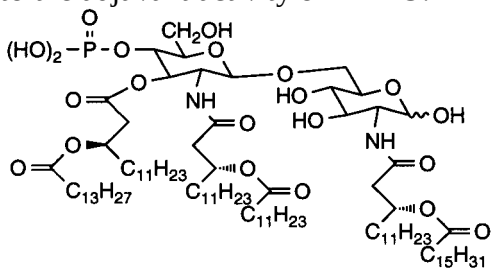
- Jones, W. R. *et al*., 1988, Phase I clinical trial of world health organization birth control vaccine. *The Lancet*, 1:1295-1298.
- Jones, G. L. *et al*., 1990, Peptide vaccines derived from a malarial surface antigen: effects of dose and adjuvants on immunogenicity. *Immunology letters* 24:253-260.
- Elliot, S. *et al*., 1994, Human compatible adjuvant induces protective cytotoxic T lymphocytes JIM peptide vaccine - Proceedings in CHI Vaccines - New Technologies and Applications -Alexandria VA, March 21-23.

CONTACT(S): SEPPIC, 75321 Paris Cedex 07, France, Ph: 33140 62 57 30; Fax: 33140 62 52 53.

COMPONENT/ADJUVANT NAME: MPL™

OTHER NAME(S): 3-Q-desacyl-4'-monophosphoryl lipid A; 3D-MLA

STRUCTURE: MPL™ is composed of a series of 4'-monophosphoryl lipid A species that vary in the extent and position of fatty acid substitution. The hexaacyl structure shown below is the most highly acylated and most abundant component in MPLO. Species with five and four fatty acids are also present. All structures contribute to the adjuvant activity of MPLO.



SOURCE: Derived from the lipopolysaccharide (LPS) of *Salmonella minnesota* R595. Obtained by treatment of LPS with mild acid and base hydrolytic conditions, and chromatographic purification of the resulting 3D-MLA.

USES: As a primary adjuvant in adjuvant formulations. Adjuvant activity is manifested either alone in aqueous solution with antigen, or in combination with particulate vehicles (e.g., oil-in-water emulsions). Activity may be enhanced by use of vehicle that enforces close association with antigen.

APPEARANCE: Colorless, odorless white powder.

MOLECULAR WEIGHT: 1540-1670 (average).

RECOMMENDED STORAGE: Indefinite stability as Iyophilized powder (in excess of 5 years if stored at 5'C). Available data indicates stability in aqueous solution is maximum between pH 5-6. An aqueous formulation was stable (<10% loss of most highly acylated component) for the equivalent of 2-3 years in an accelerated stability study.

CHEMICAL/PHYSICAL PROPERTIES: Composed of closely related 4'-monophosphoryl lipid A species that vary only in terms of fatty acid content (see above). All species in MPLO are highly amphiphilic. MPLO is probably aggregated in aqueous solution at concentrations above -1 nM. Micelles or liposomes are formed depending on excipients and conditions of formulation. Solubility in water is greatest above pH 7, and is diminished below pH 5. Surfactants enhance solubility in water. Soluble in oils (e.g., squalene).

INCOMPATIBILITY: Solubility is diminished significantl in the presence of divalent metal cations.

SAFETY/TOXICITY: Has been studied in human phase I/II clinical trials. Results to date indicate that MPLO is well tolerated at doses that exhibit beneficial immunostimulating activities. MPLO is pyrogenic at high doses.

- Rudbach, J. A. et al. Prophylactic use of monophosphoryl lipid A in patients at risk for sepsis, in: Bacterial Endotoxins: Basic Science to Anti-Sepsis Strategies - Proceedings of the International Conference on Endotoxins IV (J. Levin, A. Sturk, T. Van Der Poll, and S.J.H. Van Deventer, eds), John Wiley, New York (in press).

- Thoelen, S. et al. Immunogenicity of a recombinant hepatitis B vaccine with monophosphoryl. lipid A administered following various two-dose schedules, Abst. 340, 33rd Intersci. Conf. of Antimicrobial Agents and Chemother., Oct., p. 182 (1993).antiumoral ane
- Van Damme, -P. et al S * ety, t d cellular immunify~ 6t recombinant hepatitis B vaccine with monophosphoryl lipid A in healthy volunteers, Abst. 667, 33rd Intersci. Conf. ofAntimicrobial Agents and Chemother., Oct., p. 241 (1993).

ADJUVANT PROPERTIES: Numerous references have documented the adjuvant activity of MPL0.

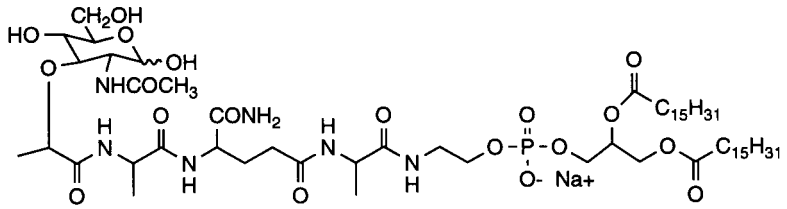
- Ulrich, J. T. *et al*. The adjuvant activity of monophosphoryl hpid A in: Topics in Vaccine Adjuvant Research Spriggs, D.R. and Koff, W.C., Eds. CRC Press, Boston, MA, pp. 133-143 (1991).
- Ivins, B. E. *et al*. Immunization against anthrax with *Bacillus anthracis* protective antigen combined with adjuvants. Infect. Immun. 60: 662-668 (1992).
- Gustafson, G. L. and Rhodes, M. J. Bacterial cell wall products as adjuvants: Early interferon gamma as a marker for adjuvants that enhance protective immunity. Res. Immunol. 143:483-488 (1992).
- Baker, P. J. Regulation of magnitude of antibody response to bacterial polysaccharide antigens by thymus-derived lymphocytes. Infect. Immun. 58: 3465-3468 (1990).
- Ulrich, J. T. and Myers, K. R. Monophosphoryl. Lipid A as an adjuvant: Past experience and new directions, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York (in press, 1994).

CONTACT(S): J.T. Ulrich/K.R. Myers, Ribi ImmunoChern Research, Inc., Hamilton, MT 59840, Ph: 406-363-6214; Fax: 406-363-6129; E-mail: 74043.1020@compuserve.com

COMPONENT/ADJUVANT NAME: MTP-PE

OTHER NAME(S): N-acetyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1,2-dipalmitoyl-sn-glycero-3-(hydroxy-phosphoryloxy)) ethylamide, mono sodium salt.

STRUCTURE:



SOURCE: Chemical synthesis by Ciba-Geigy Ltd., Basel, Switzerland.

USES: Immunomodulator. Optionally a part of MF59.

APPEARANCE: White powder.

MOLECULAR WEIGHT: 1313.55

RECOMMENDED STORAGE: 2-8° C

CHEMICAL/PHYSICAL PROPERTIES: Amphiphilic properties. Solubility in water 1%. Biodegradable.

INCOMPATIBILITY: Hydrolysis at high or low pH values.

SAFETY/TOXICITY: MTP-PE has been injected intravenously in a liposomal formulation in cancer patients and was safe up to 6 mg/m^2. Addition of MTP-PE to the MF59 adjuvant results in increased rates of local and systemic reactions over those seen in the absence of the muramyl peptide. No evidence of uveitis was seen in any patients receiving MF59 with MTP-PE.

- Wintsh, J., *et al*, 1991, Safety and immunogenicity of a genetically engineered human immunodeficiency virus vaccine, *J. Infect. Dis.*, 163:219.

ADJUVANT PROPERTIES: In seronegative populations, humoral and cellular responses to HSV and HIV vaccine were not enhanced when MTP-PE was included in MF59. The addition of MTP-PE to the MF59-based HIV vaccine in HIV seropositive individuals resulted in a marked increase in HIV antigen lymphocyte proliferation.

- Sanchez-Pestador, L., *et al*., 1988, The effect of adjuvants on the efficacy of a recombinant herpes simplex glycoprotein vaccine, *J. Immunol*., 141:1720-1727.
- Van Nest, G. A., *et al*, 1992, Advanced adjuvant formulations for use with recombinant subunit vaccines in: Vaccines 92, Ed by F. Brown, R,M. Chanock, H. S. Ginsberg and R. A. Lemer, Cold Spring Harbor Press, Plainview, NY, pg 57.
- Van Nest, G. A. *et al*., 1995, MF59: Design and evaluation of a safe and potent adjuvant for human vaccines, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

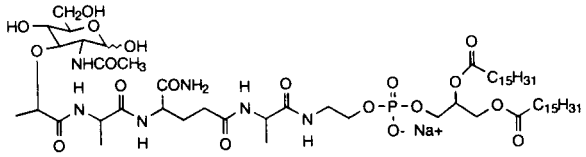
CONTACT(S): Dr. Peter van Hoogevest, Ciba-Geigy Ltd., CH-4002 Basel, Switzerland, Ph: 41-61

6965651, Fax: 41-61-696-6981, Also: Gary Ott, Chiron Corp., Emeryville, CA Ph: 510-923-2964; Fax: 510-923-4265.

COMPONENT/ADJUVANT NAME: MTP-PE Liposomes

OTHER NAME(S): MTP-PE Antigen presenting liposomes

STRUCTURE:



SOURCE: Chemical synthesis.

- Phillips, N. C. *et al*., 1985, Activation of aleveolar macrophage tumoricidial activity and eradication of experimental metastases by freeze-dried liposomes containing a new lipophilic muramyl dipeptide derivative. *Cancer Res*., 45:128-134.

USES: Primary adjuvant, liposome formulated sustained release form, co-emulsified with antigen and lipids.

APPEARANCE: Lyophilized white powder.

MOLECULAR WEIGHT: MTP-PE, 1313.55

RECOMMENDED STORAGE: Store at 4° C for both the parent lyophilized compound and the liposome-formulated MTP-PE.

CHEMICAL/PHYSICAL PROPERTIES: Amphipathic molecule with good water solubility (1-2 mg/mL). It may form as mixed micelle suspension at high concentration. MTP-PE-liposomes are multilamellar in nature, ranging from 1-5 um in diameter.

INCOMPATIBILITY: Unknown

SAFETY/TOXICITY: Liposome-formulated MTP-PE is currently under phase II/III trials. Some systemic toxicity with MTP-PE given with gp120 based HIVA vaccines.

ADJUVANT PROPERTIES:

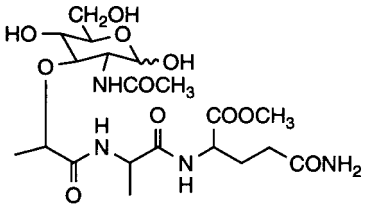
- Ho, R. J. Y. *et al*., 1989, Antigen presenting liposomes are effective in treatment of recurrent herpes simplex genitalis in guinea pigs. *J. Virol*., 63:2951-2958.
- Ho, R. J. Y. *et al*., 1994, Disposition of antigen-presenting liposomes in vivo: Effect on presentation of HSV antigen rgD. *Vaccine*, 12:235-242.
- Bui, T. *et al*. Effect of MTP-PE liposomes and IL-7 on induction of antibody and cell-mediated immune responses to a recombinant HIV envelope protein. *J. AIDS*, 7:799-806.

CONTACT(S): Rodney J. Y. Ho, Department of Pharmaceutics, University of Washington, Seattle, WA 98195, Ph: 206-543-9434; Fax: 206-543-3204, Also: Dr. Peter van Hoogevest, Ciba-Geigy Ltd., CH4002 Basel, Switzerland, Ph: 41-61-6965651, Fax: 41-61-696-6981.

COMPONENT/ADJUVANT NAME: Murametide

OTHER NAME(S): Nac-Mur-L-Ala-D-Gln-OCH3

STRUCTURE:



SOURCE: Synthesis.

USES: Administered in water-in-oil (w/o) emulsion as adjuvant of humoral and cell-mediated immunity.

APPEARANCE: White powder.

MOLECULAR WEIGHT: 506.5

RECOMMENDED STORAGE: Stored as a powder at 4º C. Protect from light and humidity. Stable for more than 5 years.

CHEMICAL/PHYSICAL PROPERTIES: Hydrophilic molecule. Freely soluble in water. The solution is clear, colorless and odorless, store at pH 5 to 7.5.

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: Acute, subacute and chronic toxicity performed in rats and monkeys allow administration in clinical studies of 1 to 4 s.c. injections at 2-4 week intervals at doses of 35-100 µg/kg. Pharmacokinetics was performed in rats and dogs. A Phase I clinical trial was completed that showed no toxicity at dosages up to 150 µg/kg.

ADJUVANT PROPERTIES: When administered in saline, Murametide is non pyrogenic, induces granulocytosis and enhances the humoral response. Murametide displays the same profile of adjuvant activity as MDP and has been chosen for development because of its favorable therapeutic ratio. When administered in 50% water-in-oil emulsion, it mimics the activity of Freund's complete adjuvant without its side effects. US Patent #4693998.

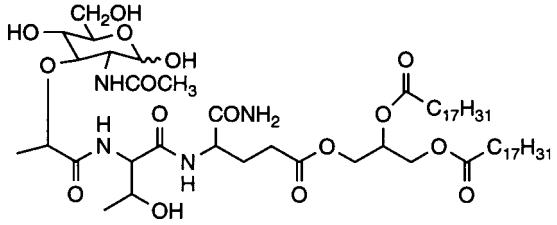
- Audibert, *et al*., 1985, Muramyl peptides as immunopharmacological response modifiers in: Biological Response Modifiers, P. F. Torrence, Ed., Academic Press, Inc. Orlando, Fl, pp 307-37.

CONTACT(S): Prof. Louis Chedid, Dr. Francoise Audibert, VACSYN S.A., 75015 Paris, France, Ph: 331-40-60-75-92; Fax: 331-40-60-75-73.

COMPONENT/ADJUVANT NAME: Murapalmitine

OTHER NAME(S): Nac-Mur-L-Thr-D-isoGIn-sn-glyceroI dipalmitoyl

STRUCTURE:



SOURCE: Synthesis.

USES: Administered in water-in-oil (w/o) emulsion as adjuvant of humoral and cell-mediated responses.

APPEARANCE: White powder.

MOLECULAR WEIGHT: 1072

RECOMMENDED STORAGE: Stored as a powder at 4º C. Protect from light and humidity.

CHEMICAL/PHYSICAL PROPERTIES: Lipophilic molecules giving homogeneous suspensions in mineral oil.

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: Acute toxicity in mice, rats, rabbits and guinea pigs satisfactorily completed. Preliminary subacute toxicity in dogs satisfactorily completed. Further toxicology studies in process.

ADJUVANT PROPERTIES: Lipophilic MDPs are more active than hydrophilic MDPs. In saline, they are strong immunoadjuvants of humoral immunity and weaker adjuvants of cell-mediated immunity. In 50% w/o emulsions, they are strong immunoadjuvants and mimics Freund's complete activity. In contrast with other molecules of this subgroup, Murapalmitine is devoid of side effects and thus has been chosen for further development. U.S. Patent #4939122 and 5210072.

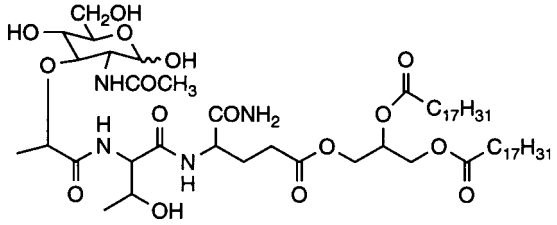
- Audibert F., *et al*., 1985, Muramyl peptides as immunopharmacological response modifiers in: Biological Response Modifiers, P. F. Torrence, Ed., Academic Press, Inc. Orlando, FL pp 307-37.

CONTACT(S): Prof. Louis Chedid, Dr. Francoise Audibert, VACSYN S.A., 75015 Paris, France, Ph: 331-40-60-75-92; Fax: 331-40-60-75-73.

COMPONENT/ADJUVANT NAME: D-Murapalmitine

OTHER NAME(S): Nac-Mur-D-Ala-D-isoGln-sn-glycerol dipalmitoyl

STRUCTURE:



SOURCE: Synthesis.

USES: Administered in water-in-oil (w/o) emulsion as adjuvant of humoral and cell-mediated immunity. To be developed as an adjuvant for vaccines likely to contain autoantigens.

APPEARANCE: White powder.

MOLECULAR WEIGHT: 1056

RECOMMENDED STORAGE: Stored as a powder at 4º C. Protect from light and humidity.

CHEMICAL/PHYSICAL PROPERTIES: Lipophilic molecules giving homogeneous suspensions in mineral oil.

INCOMPATIBILIT'Y: Avoid strong bases.

SAFETY/TOXICITY: This MDP analog is non-pyrogenic in rabbits and displays no acute toxicity when tested in mice, rats, guinea pigs, and rabbits. Further toxicological studies will be performed.

ADJUVANT PROPERTIES: D-Murapalmitine is a strong adjuvant of humoral and cell-mediated immunity when administered in a 50% mineral oil emulsion. However, in contrast with other MDPs, it does not induce experimental allergic encephalomyelitis (EAE) when administered with myelin basic protein. When given with an antigenic preparation containing hetero and autologous epitopes, it favors the response to the heterologous determinants. US patent #4939122, French patent #9207126.

F. Audibert, *et al*., 1985, Muramyl peptides as immunopharmacological. response modifiers in: Biological Response Modifiers, P. F. Torrence, Ed., Academic Press, Inc. Orlando, FL, pp 307-37.

CONTACT(S): Prof. Louis Chedid, Dr. Francoise Audibert, VACSYN, S. A., 75015 Paris, France, Ph: 331-40-60-75-92; Fax: 331-40-60-75-73.

COMPONENT/ADJVVANT NAME: NAGO

OTHER NAME(S): Neuraminidase-galactose oxidase

STRUCTURE: NAGO is a mixture of the two enzymes- neuraminidase and galactose oxidase Ag 1:5 ratio in units of activity. The primary amino acid sequences of the two enzymes are appended.

- McPherson, M. J. *et al*., 1992, Galactose oxidase of *Dactylium denroides:* Gene cloning and sequence analysis. *J. Biol. Chem.*, 267:8146-8152.
- Galen, J. E. *et al*., 1992, Role of *Vibrio cholerae* neuraminidase in the function of cholera toxin. Infection and Immunity, 60:406-415.

Sequence of Neuraminidase (E.C. 3.2.18), NA:
MRFKNVKKTALMLAMFGMATSSNAALFDYNATGDTEFDSPAKQGWMQDNTNNGSGVLTNA
DGMPAWLVQGIGGRAQWTYSLSTNQHAQASSFGWRMTTEMKVLSGGMITNYYANGTQRVLPIIS
LDSSGNLVVEFEGQTGRTVLATGTAATEYHKFELVFLPGSNPSASFYFDGKLIRDNIQPTASKQNMI
VWGNGSSNTDGVAAYRDIKFEIQGDVIFRGPDRIPSIVASSVTPGVVTAFAEKRVGGGDPGALSNTN
DIITRTSRDGGITWDTELNLTEQINVSDEFDFSDPRPIYDPSSNTVLVSYARWPTDAAQNGDRIKPW
MPNGIFYSVYDVASGNWQAPIDVTDQVKERSFQIAGWGGSELYRRNTSLNSQQDWQSNAKIRIVD
GAANQIQVADGSRKYVVTLSIDESGGLVANLNGVSAPIILQSEHAKVHSFHDYELQYSALNHTTTL
FVDGQQITTWAGEVSQENNIQFGNADAQIDGRLHVQKIVLTQQGHNLVEFDAFYLAQQTPEVEK
DLEKLGWTKIKTGNTMSLYGNASVNPGPGHGITLTRQQNISGSQNGRLIYPAIVLDRFFLNVMSIYS
DDGGSNWQTGSTLPIPFRWKSSSILETLEPSEADMVELQNGDLLLTARLDFNQIVNGVNYSPRQQFL
SKDGGITWSLLEANNANVFSNISTGTVDASITRFEQSDGSHFLLFTNPQGNPAGTNGRQNLGLWFS
FDEGVTWKGPIQLVNGASAYSDIYQLDSENAIVIVETDNSNMRILRMPITLLKQKLTLSQN

Sequence of Galactose Oxidase (E.C. 1.1.3.9), GO:
MKHLLTLALCFSSINAVAVTVPHKAVGTGIPEGSLQFLSLRASAPIGSAISRNNWAVTCDSAQSGNE
CNKAIDGNKDTFWHTFYGANGDPKPPHTYTIDMKTTQNVNGLSMLPRQDGNQNGWIGRHEVYL
SSDGTNWGSPVASGSWFADSTTKYSNFETRPARYVRLVAITEANGQPWTSIAEINVFQASSYTAPQP
GLGRWGPTIDLPIVPAAAAIEPTSGRVLMWSSYRNDAFGGSPGGITLTSSWDPSTGIVSDRTVTVTK
HDMFCPGISMDGNGQIVVTGGNDAKKTSLYDSSSDSWIPGPDMQVARGYQSSATMSDGRVFTIGG
SWSGGVFEKNGEVYSPSSKTWTSLPNAKVNPMLTADKQGLYRSDNHAWLFGWKKGSVFQAGPST
AMNWYYTSGSGDVKSAGKRQSNRGVAPDAMCGNAVMYDAVKGKILTFGGSPDYQDSDATINA
HIITLGEPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGGQRRGIPFEDSTPVFTPEIYVPEQDTFY
KQNPNSIVRVYHSISLLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLYNSNGNLATRPKITRTST
QSVKVGGRITISTDSSISKASLIRYGTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGVALPGYWM
LFVMNSAGVPSVASTIRVTQ

SOURCE: GO is biochemically purified from *Dactylium dendroides.* It has an activity of approximately 800 units per mg of protein and is commercially available from Sigma Chemical Co. (Cat No. G3385) as a partially purified lyophilized powder containing 25% protein to be reconstituted in buffered saline. NA is biochemically purified from *Vibrio cholerae.* It has an activity of 25 units per µg of protein and is commercially available from Merck Ltd. (BDH Laboratory Supplies) Merck House, Poole, Dorset BH12 1BR UK.

USES: Primary adjuvant. A mixture of the two enzymes containing 10 units NA & 50 units of GO per 1 ml, of aqueous solution and antigen is administered subcutaneously or intramuscularly. It is comparable in effectiveness to Freund's complete adjuvant, but is non-reactogenic. It is especially effective in the induction of CD8 it toxic T-cell responses.

APPEARANCE: GO - Lyophilized off-white solid. NA - clear, colorless aqueous solution.

MOLECULAR WEIGHT: GO - 68,500. NA - 83,000.

RECOMMENDED STORAGE: Long term storage: GO at -20º C, NA at 40º C.

CHEMICAL/PHYSICAL PROPERTIES: Biologically active proteins in aqueous media.

INCOMPATIBILITY: Avoid denaturing conditions. Not compatible with alum. Activity reduced in oil emulsions.

SAFETY/TOXICITY: Histopathology studies in mice showed that NAGO is less inflammatory than alum and produced no adverse local or systemic reactions.

ADJUVANT PROPERTIES: NAGO generates cell surface Schiff base-forming aldehydes on antigen presenting cells and Th-cells, thereby amplifying physiologic Schiff base formation that occurs between cell-surface ligands as an essential element in APC:T-cell inductive interaction. It is a potent non-inflammatory adjuvant with viral, bacterial and protozoal subunit vaccines, and is especially effective in the generation of cytotoxic T-cells.

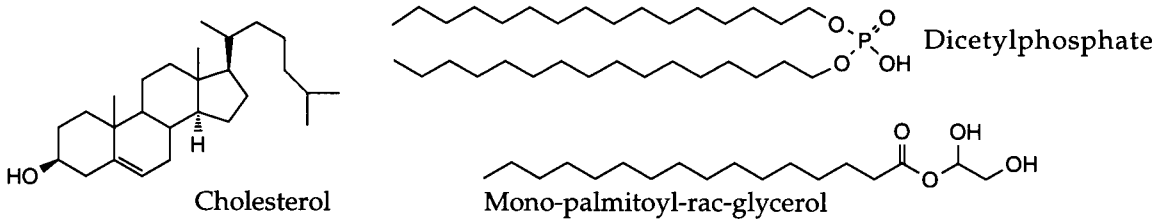
- Zheng, B. *et al*., 1992, Glactose oxidation in the design of immunogenic vaccines. *Science*, 256:1560-1563.
- Zhong, G. *et al*., 1993, Immunogenicity evaluation of a lipidic amino acid based synthetic peptide vaccine for *Chlamydia trachomatis. J. Immunol*., 151:3728-3736 (1993).

CONTACT(S): Dr. John Rhodes, Wellcome Foundation Ltd., Beckenham. Kent UK., BR3 3BS, Ph: 44-81-639-5336; Fax: 44-81-663-6176.

COMPONENT/ADJUVANT NAME: Non-Ionic Surfactant Vesicles

OTHER NAME(S): NISV

STRUCTURE: Multi-lamellar vesicles comprising a mixture of non-ionic surfactant (e.g., 1-monopalmitoyl-rac-glycerol), cholesterol and dicetyl phosphate.



SOURCE: Synthetic/semi-synthetic.

- Alexander, J. and Brewer, J. M., Vaccines containing non-ionic surfactant vesicles, PCT/GB93/00716, priority date 7 April 92.

USES: Used as a primary vaccine adjuvant for entrapped antigen. NISV adjuvant biodegrades in vivo with release of the entrapped antigen. NISV adjuvant induces humoral and cell-mediated immunity and probably functions by targeting the antigen to the macrophage population.

APPEARANCE: Milky, colloidal suspension.

MOLECULAR WEIGHT: 1-Monopalmitoyl glycerol: 329, cholesterol: 386, dicetyl phosphate: 547.

RECOMMENDED STORAGE: Component raw materials should be stored at low humidity. Refrigeration of NISV at 4º C is preferred for antigen-containing preparations. Optimal storage conditions are under evaluation.

CHEMICAL/PHYSICAL PROPERTIES: Stable at neutral and alkaline pH. Components are amphiphilic, insoluble in water and soluble in chloroform. The T_c of NISV is approximately 55º C. NISV are formulated as a suspension in saline.

INCOMPATIBILITY: Incompatible with most organic solvents and some detergents; osmotically sensitive.

SAFETY/TOXICITY: Extremely low toxicity of NISV has been demonstrated in rat studies after administration by both the subcutaneous or intramuscular route. At doses up to 575 mg/kg body weight there was no persistence of NISV at the site of injection (s.c.).

- Brewer, J. M., *et al.*, 1994, Non-ionic surfactant vesicles as vaccine delivery systems, in: Proceedings of the Second Conference on Industrial Immunology (112), July, 1994, Brighton, Chameleon Press Ltd., London, UK pp 34-36.

ADJUVANT PROPERTIES: Induces both a humoral and cell-mediated immune response. Preferentially stimulates the Th1 sub-population of T-helper cells. Effective with antigens within a broad size range, from short peptides to particulates. Adjuvant function is unrestricted by genetic background.

- Brewer, JM and Alexander, J., 1994, The adjuvant activity of non-ionic surfactant vesicles (niosomes) on the BALB/c humoral response to bovine serum albumin. *Immunology*, 75:570-575.

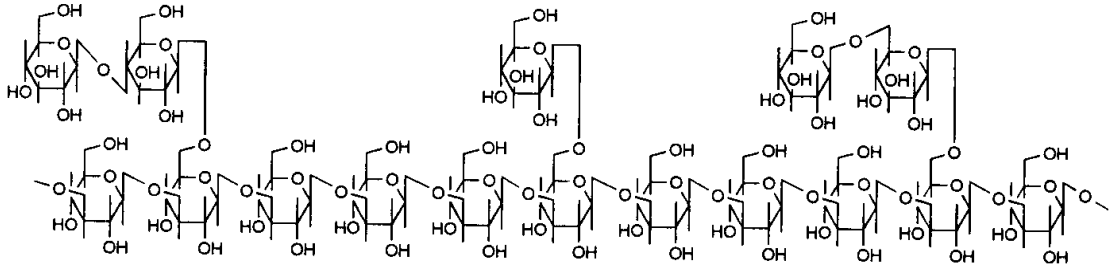
- Brewer, JM and Alexander, J., 1994, Studies on the adjuvant activity of non-ionic surfactant vesicles: adjuvant-driven IgG2a production independent of MHC control. *Vaccine*, 12: 613-619.
- Brewer, J.M. et al, The demonstration of an essential role for macrophages in the in vivo generation of IgG2a antibodies. Clin Exp Immunol. 1994 97:164-171.

CONTACT(S): Jurek S. Sikorski, Proteus Molecular Design Limited, Macclesfield, Cheshire SKI 1 0JL, UK. Ph: 44-625-500555; Fax: 44-625-500666.

COMPONTENT/ADJUVANT NAME: Pleuran

OTHER NAME(S): β-glucan; glucan

STRUCTURE: A β-1,3-linked glucose polymer having β-D-glucosyl side chains attached by alternate β(1,6) or β(1,4) bonds at the -0-6 position of every fourth anhydroglucose unit.



SOURCE: Isolated from the fruit-body of the oyster fungus *Pleurotus ostreatus* by alkali extraction at 95-100º C, followed by bleaching with sodium chlorite (pH 3.5-4.5) at 50-60º C. The bleached products were washed in water, dehydrated in organic solvent, and finally dried by vacuum at 60º C.

- Kuniak, L., *et al*., 1993, A new fungal glucan and its preparation. W.I.P.O. Patent No. W093/12243.
- Karacsonyi, S., and Kuniak, L. Polysaccharides of Pleurotus ostreatus: Isolation and structure of Pleuran, an alkali-insoluble β-D-glucan, *J. Biopolymers* (in press).

USES: Administered with antigen for enhancement of both humoral and cell-mediated immunity. β-glucans exert their immunostimulatory activities by binding to specific P-glucan receptors on macrophages. This ligand-receptor interaction results in macrophage activation and, in certain formulations, promotes antigen targeting.

- DiLuzio, N.R., *et al*., 1979, Evaluation of the mechanism of glucan-induced stimulation of the reticuloendothelial system, *J. Reticuloendothel. Soc*. 7:731-742.
- Czop, J.K., and Austen, K.F., 1985, A β-glucan inhibitable receptor on human monocytes: Its identity with the phagocytic receptor for particulate activators of the alternative compliment pathway, J. Immunol. 134:2588-2593.

APPEARANCE: White, odorless powder. Viscous in aqueous solution.

MOLECULAR WEIGHT: MW = 762 kD.

RECOMMENDED STORAGE: Stable to light. Store solid Pleuran at room temperature and aqueous suspensions at 4'C. Optimal storage conditions are to be determined.

CHEMICAL/PHYSICAL PROPERTIES: Water insoluble. Median particle size of homopolymer is 150 um. Purified preparations contain <0.57% chitin and <0.03% protein.

INCOMPATIBILITY: Alkaline pH disrupts the triple helical conformation.

SAFETY/TOXICITY: In preclinical studies, Pleuran has been intravenously administered at doses up to 25 mg/kg body weight and was well tolerated. Human clinical trials of β-glucans isolated from either plants or microorganisms indicate the feasibility of administering these compounds to humans without toxicity. Glucan particles bioerode over time in a physiological environment.

- Mansel, P.W.A. *et al*., 1975, Macrophage-mediated destruction of human malignant cells in vivo, *J. Natl. Cancer Inst*. 54:571-580.
- Okamura, K. *et al*., 1986, Clinical evaluation of Schizophyllan combined with irradiation in patients with cervical cancer, *Cancer* 58:865-872.
- Chihara, G. *et al*., 1989, Lentinan as a host defense potentiator (HDP), *Int. J. Immunother*. 4:145-154.
- Ostroff, G.R., 1994, Future therapeutic applications of Betafectin, a carbohydrate-based immunomodulator, The Second Annual Conference on Glycotechnology.

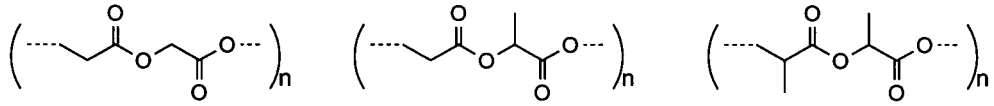
ADJUVANT PROPERTIES: Rabbits as well as mice immunized once by coadministration of viral antigens and 60 µg of Pleuran produced at least 20-fold higher antibody titers than control animals injected with the immunogen alone.

CONTACT(S): Richard McIntosh, Genesis Technology Group, Inc., Cambridge, MA 02139. Ph: 617576-6610; Fax: 617-876-4002. Also: Dr. Nahid Mohagheghpour, SRI International, Menlo Park, CA 94025. Ph: 415-859-3516; Fax: 415-859-3342.

COMPONENT/ADJUVANT NAME: PLGA, PGA, and PLA

OTHER NAME(S): Homo-and co-polymers of lactic and glycolic acid; Lactide/glycolide polymers; poly-lactic-co-glycolide

STRUCTURE: Structures shown below (left to right): PGA, homo-PLGA, and PLA.



SOURCE: Synthesized by the ring opening polymerization of the cyclic dimers, lactide and glycolide.

- Deasy, P. B. *et al*., 1989, Preparation and characterization of lactic/glycolic acid polymers and copolymers. *J. Microencapsul*., 6:369-378.

USES: Antigens incorporated in PLGA microspheres have exhibited enhanced and prolonged antibody activity responses compared to equivalent doses of free antigen.

APPEARANCE: Odorless, white to tan pellets.

MOLECULAR WEIGHT: Standard grades available from 10,000 to 500,000.

RECOMMENDED STORAGE: Store at 0º C or below and minimize exposure to moisture to maintain quality.

CHEMICAL/PHYSICAL PROPERTIES: Stable except in presence of moisture. Polymers react with water and degrade to glycolic and/or lactic acid.

INCOMPATIBILITY: Reacts with water and aqueous acids and bases. Hydrolyzes to form hydroxyacetic acid (glycolic acid) and lactic acid.

SAFETY/TOXICITY: Materials are used commercially as surgical suture, staples and clips and sustained release delivery systems. FDA has approved specific applications using this family of polymers. Drug Master File (DMF) established with FDA.

ADJUVANT PROPERTIES: The adjuvant properties of polylactides have been ascribed to the - e of the microspheres and small microspheres (<10 gm) may be phagocytosed to enhance antigen presentation. However, the major use of polylactides for vaccine delivery is based upon their ability to control the release of antigen after administration, thereby eliminating or reducing the need for boost immunizations.

- Cleland, J., *et al*., 1994, Development of a single shot subunit vaccine for HIV-1, AIDS Res Hum Retroviruses. 1994; 10 Suppl 2: S21-S26.
- Eldridge, J. H., *et al*., 1991,Biodegradable and biocompatible poly(DL-lactide-co-glycohde) microspheres as an adjuvant for staphylococcal enterotoxin B toxoid which enhances the level of toxin-neutralizing antibodies, *Infect. Immun.* 59: 2978-2986.
- Eldridge, J. H., *et al*., 1991, Biodegradable microspheres as a vaccine delivery, *Mol. Immunol.* 28: 287-294.
- Singh, M., *et al*., 1992, Immunogenicity studies on diphtheria toxoid loaded biodegradable microspheres, *Int. L Pharm.* 85: R5-R8.
- Hazrati, A. M. *et al*., 1993, Studies of controlled delivery tetanus vaccine in mice. *Proc. Intern. Symp, Control. Rel. Bioact. Mater.*, 20:67-68.

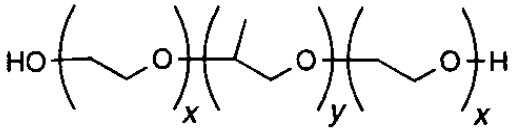
- Hazrati, A. M. *et al*., 1993, *Salmonella enteritidis* vaccine utilizing biodegradable microspheres. *Proc. Intern. Symp. Control. Rel. Bioact. Mater*., 20:101-102.
- Aguado M. T. and Lambert, P.-H., 1992, Controlled-release vaccines-biodegradable polylactide/polyglycolide (PL/PG) microspheres as antigen vehicles. *Immunobiol*. 184:113-125.
- Esparza, I. and Kissel, T., 1992, Parameters affecting the immunogenicity of microencapsulated tetanus toxoid. *Vaccine*, 10:176-180.
- Nellore, R. V. *et al*., 1992, Evaluation of biodegradable microspheres as vaccine adjuvant for hepatitis B surface antigen. *J. Parenter. Sci. Tech*., 46:176-180.
- Langer, R. *et al*., 1995, Polymeric systems for vaccine delivery, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
- Cleland, J. L., 1995, Design and production of single irrununization vaccines using polylactide polyglycolide systems, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): There are several suppliers of PLGA polymers. This monograph prepared by: Medisorb Technologies Intl. L. P., Cincinnati, OH 45242, Ph: 800-772-5091; Fax: 513-489-7244. Also: Jeffrey L. Cleland, Genentech, Inc. South San Francisco, CA 94080, Ph: 415-225-3921; Fax: 415-225-2866; Email: cleland.jeffrey@gene.com

COMPONENT/ADJUVANT NAME: Pluronic L121

OTHER NAME(S): Poloxamer 401

STRUCTURE:



SOURCE: Synthetic block copolymer of ethylene oxide and propylene oxide.

USES: Component of IDEC Antigen Formulation (AF) present in final concentration of 0.05-1.25% w/v with antigen, and as a component of the Syntex Adjuvant Formulation (SAF) present in a final concentration of 2.5% (w/v) with antigen.

APPEARANCE: Off-white viscous liquid at room temperature.

MOLECULAR WEIGHT: Approximately 4400.

RECOMMENDED STORAGE: Airtight container at room temperature.

CHEMICAL/PHYSICAL PROPERTIES: Water insoluble surfactant with hydrophilic/lipophilic balance (HLB) of approximately 1.0 which classifies the compound as a spreading agent.

INCOMPATIBILITY: None found.

SAFETY: Currently under study.

ADJUVANT PROPERTIES: The amphipathic structure is hypothesized to enhance the presentation of antigen to cells of the immune system. Also see references for SAF-1 and Antigen Formulation.

Allison, A. C. and Byars, N. E. Syntex adjuvant formulation. Res. Immunol., 143:519-525 (1992).

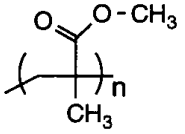
- Hunter, R.L. *et al*., 1981, The adjuvant activity of nonionic block polymer surfactants I. The role of hydrophile-lipophile balance, *J. Immunol*., 127:1244-1250.
- Hunter, R. L. *et al*., 1984, The adjuvant activity of nonionic block polymer surfactants II. Antibody formation and inflammation related to the structure of triblock and octablock copolymers, *J. Immunol*., 133:3167-775.
- Hunter, R. L. *et al*., 1986, The adjuvant activity of nonionic block polymer surfactants III. Characterization of selected biologically active surfaces, *Scand. J. Immunol*., 23:287-300.
- Lidgate, D. M. and Byars, N., 1995, Development of an emulsion based muramyldipeptide adjuvant formulation for vaccines, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Thomas Ryskamp, IDEC Pharmaceuticals Corporation, San Diego, CA 92121, Ph: 619-550-8500; Fax: 619-550-8750, Internet: tryskamp@idec.com.

COMPONENT/ADJUVANT: PMMA

OTHER NAMES: Polymethyl methacrylate

STRUCTURE:



SOURCE: Emulsion polymerization of methyl methacrylate

USES: Primary adjuvant for all types of antigens. Added to the aqueous antigens in concentrations of 0.05% to 1.0% (wt/wt). Optimal adjuvant concentration in most cases 0.5%.

APPEARANCE: White odorless powder; forms a white milky suspension in water.

MOLECULAR WEIGHT. 30-400 kD, depending on polymerization conditions.

RECOMMENDED STORAGE: Room temperature in solid powder form; between 2-8º C in aqueous suspension (pH range 2-11).

CHEMICAL/PHYSICAL PROPERTIES: Insoluble polymer, suspendable in aqueous solution. Forms a milky suspension upon dispersion in water, easy to resuspend once hydrated. Polymer particle size 100-500 nm.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: Poly(methyl methacylate) has been used as an artificial bone material and bone cement in humans for over fifty years. Breakdown of these artificial bone materials leads to fragments with similar particle size to that of the adjuvant; no adverse reactions have been observed.

ADJUVANT PROPERTIES: Good adsorbate for a large number of antigens, particularly hydrophobic antigens. Antigen may be absorbed to previously polymerized particles, or may be incorporated into the polymer particles by polymerization in the presence of the antigen. PMMA is slowly biodegradable (40%/yr in rats). PMMA enhances the temperature stability of an number of antigens.

- Kreuter, J., 1992, Physicochemical characterization of nanoparticles and their potential for vaccine preparation, *Vaccine Res.*, 1:93-98.
- Kreuter, J. *et al*., 1981, Long-term studies of microcapsulated and adsorbed influenza vaccine nanoparticles. *J. Pharm. Sci.*, 70:367-71.
- Kreuter, J., 1995, Nanoparticles as adjuvants for vaccines, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Dr. Jorg Kreuter, Institut für Pharmazeutische Technologie, J. W. Goethe-University, D-60439 Frankfurt, Germany Ph: 49-69-5800-9682; Fax: 49-69-5800-9694.

COMPONENT/ADJUVANT NAME: PODDS™

OTHER NAMES: Proteinoid microspheres

STRUCTURE: Acylated amino acids (early experiments done with thermally condensed α-amino acids).

SOURCE: Chemical synthesis, purified by reprecipitation in acid. Microspheres are made in citric or acetic acid; additives such as gum arabic, gelatin or lactose may be added to formulate the material.

USES: Microspheres are being used as vehicles for oral immunization for the development of both mucosal and hurnoral responses. They are thought to protect antigens, target them to Peyer's patches and/or facilitate transport of the protein antigens across mucosal epithelium.

APPEARANCE: Exists as liquid suspension or free flowing powder (after lyophilization).

MOLECULAR WEIGHT: 250-300 kD.

RECOMMENDED STORAGE: If lyophilized, store at room temperature under low humidity. Stability studies of suspension have not been conducted.

CHEMICAL/PHYSICAL PROPERTIES: Proteinoids have good water solubility at neutral pH, and precipitate out as microspheres at pH 2-3 at concentrations from 20-100 mg/mL. Particle size distribution of microspheres ranges from 0.1 to 10 µm, depending on the composition and formulation. Microspheres remain stable at acid pHs, soluble at neutral pHs. Proteinoids interact noncovalently with proteins, and have high encapsulation affinities.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: Toxicology data available on thermal condensate products in rats and dogs, following acute and subacute i.v. and p.o. dosing; dosing of human volunteers with the thermal condensate carrier resulted in good safety profile. Formal toxicology not completed with acylated amino acid microspheres; these carriers have been administered orally and intraduodenally to rodents and primates at doses up to 1000 mg/kg.

ADJUVANT PROPERTIES: Serves as a vehicle for oral immunization, protecting the antigen and allowing for co-encapsulation of adjuvants with antigens in microspheres.

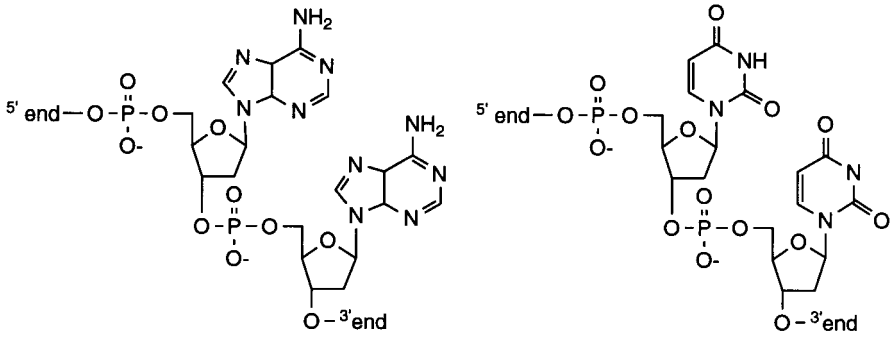
- Santiago, N. *et al*., 1993, Oral immunization of rats with proteinoid microspheres encapsulating influenza virus antigens. *Pharm. Res.*, 10:1243-1247.
- Santiago, N. *et al*., 1995, Vehicles for oral immunization, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Noemi Santiago or Robert Baughman, Emisphere Technologies, Inc., Hawthorne, NY 10532, Ph: 914-347-2220; Fax: 914-347-2498.

COMPONENT/ADJUVANT NAME: Poly rA:Poly rU

OTHER NAME(S): Poly-adenylic acid-poly-uridylic acid complex

STRUCTURE: Poly rA:poly rU is a double helix comprised of polyadenylic acid (left structure, two repeat units shown) and polyuridylic acid (right structure, two repeat units shown).



SOURCE: Synthetic. Polyribonucleotide complexes are formed following the action of the enzyme polynucleotide phosphorylase on the synthetic mononucleotide diphosphate. A hydrogen bonded double helix forms following mixing of the opposite base pairs.

USES: Immunomodulation.

APPEARANCE: White, odorless powder

MOLECULAR WEIGHT: Variable MW, ranging from 200 kD to 2,000 kD. S_{W20} values range from 4 to 11 units.

RECOMMENDED STORAGE: Stable for several years in sterile physiological saline at 4° C.

CHEMICAL/PHYSICAL PROPERTIES: Polyadenylate as potassium salt; polyuridate as ammonium salt; readily water soluble at neutral pH (pH 7.2-7.6).

INCOMPATIBILITY: Destroyed by RNase.

SAFETY/TOXICITY: No toxicity in human trials at 600 mg/m^2/wk for 6 wks.

ADJUVANT PROPERTIES: Adjuvant to humoral and cell-mediated immunity when given with antigen; increases non-specific immunity to microorganisms; antibody suppressant when given before antigen.

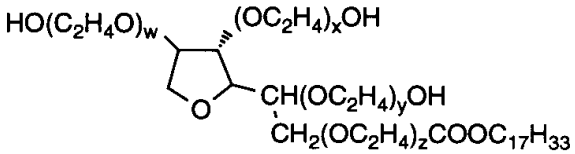
- Johnson, A. G. *et al*., 1979, Modulation of the immune system by synthetic polynucleotides. Springer Seminars h-nmunopath., 2:149-168.
- Tursz, T. A. *et al*., 1990, Poly A-poly U; An updated review, in: Immunotherapeutic Prospects, of Infectious Diseases, K. N. Mashihi and W. Lange, Eds. Springer-Verlag, Berlin, p. 263-272.
- Lacour J. *et al*., 1984, Adjuvant treatment with polyadenylic-polyuridylic acid in operable breast cancer: update results of a randomized trial. Brit. Med. J., 288 589-92.

CONTACT(S): Cynthia Ewel, Institute Henri Beaufour-USA, Washington, DC 20037, Ph: 202-9732400; Fax: 202-887-5032. Also: A. G. Johnson, University of Minnesota, Duluth, MN 55812, Ph: 218-, 726-7561; Fax: 218-726-6235.

COMPONENT/ADJUVANT NAME: Polysorbate 80

OTHER NAME(S): Tween 80; Sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl) derivatives

STRUCTURE:



SOURCE: Polysorbate 80 is produced via copolymerization of ethylene oxide with an oleate ester of sorbitan and its anhydrides.

USES: A stabilizer in the MF59 and IDEC SPT formulations, present in final concentration of approximately 0.2% w/v with antigen. Commonly used surfactant in foods, cosmetics and pharmaceuticals.

APPEARANCE: Amber, viscous liquid.

MOLECULAR WEIGHT: 1309.68

RECOMMENDED STORAGE: Airtight container at room temperature.

CHEMICAL/PHYSICAL PROPERTIES: HLB of 12-16 and therefore highly soluble in aqueous solution. The oleic acid esters are susceptible to oxidation.

INCOMPATIBILITY: Avoid strong oxidizing agents, bases and heavy metal salts.

SAFETY/TOXICITY: Mild ocular irritant (rabbit eye test 150 mg). LD50 (rat) via i.v., 1.8 g/kg, LD50 (mouse) via oral, 25 g/kg. Generally considered safe (GRAS).

ADJUVANT PROPERTIES: Polysorbate 80 has no adjuvant properties on its own. Used in emulsion vaccine formulations including MF59, SAF-1 and Antigen Formulation. See these headings for additional references.

- Sanchez-Pestador L., *et al*., 1988, The effect of adjuvants on the efficacy of a recombinant herpes simplex glycoprotein vaccine, *J. Immunol*., 141:1720-1727.
- Van Nest, G., A., et al, 1992, Advanced adjuvant formulations for use with recombinant subunit vaccines in: Vaccines 92, F. Brown, RM. Chanock, H. S. Ginsberg and R. A. Lerner, (Eds.) Cold Spring Harbor Press, Plainview, NY, pg 57.
- Van Nest, G. A. et al., 1995, MF59: Design and evaluation of a safe and potent adjuvant for human vaccines, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
- Lidgate, D. M. and Byars, N., 1995, Development of an emulsion based muramy1dipeptide adjuvant formulation for vaccines, in: Vaccine Design- M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

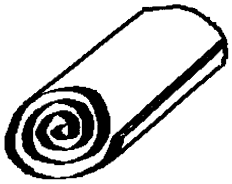
CONTACT(S): There are several suppliers of Polysorbate 80. For use in adjuvant formulations: Thomas Ryskamp, IDEC Pharmaceuticals Corporation, San Diego, CA 92121, Ph: 619-550-8500; Fax: 619-550-8750,

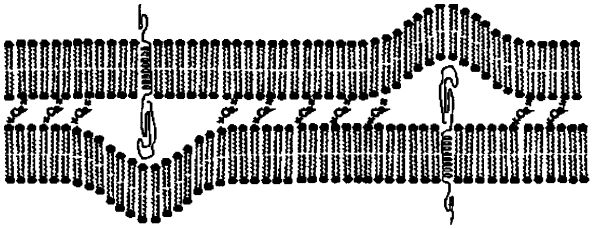
Internet: trykamp@idec.com, Also: Gary Ott, Chiron Corp., Emeryville, CA Ph: 510-923-2964; Fax: 510-293-4265.

COMPONENT/ADJUVANT NAME: Protein Cochleates

OTHER NAME(S): None

STRUCTURE: Protein cochleates are stable protein phospholipid-calcium. precipitates, which are distinct from liposomes. They have a unique structure consisting of a large, continuous, solid, lipid bilayer sheet rolled up in a spiral, with no internal aqueous space. The calcium maintains the cochleate in its rolled up form, bridging between successive layers. One of its positive charges interacts with a single negative charge on a phospholipid head group in one bilayer, and the other with a phospholipid in the opposing bilayer. Membrane proteins, or lipid-anchored peptides or proteins are tightly associated with the lipid bilayer.





SOURCE: Cholesterol, phosphatidylethanolarnine(egg or synthetic), and phosphatidylserine(bovine brain or synthetic) are obtained from Avanti Polar Lipids, Inc. Antigens which have been utilized include glycoproteins isolated directly from enveloped viruses, or expressed as recombinants in tissue culture, as well as synthetic peptides covalently linked to phosphatidylethanolamine. A mixture of phospholipids from the envelope will also be included when glycoproteins, are isolated from viruses our method of detergent extraction.

USES: Protein cochleates act as both carriers and adjuvants, providing multivalent presentation of antigens to the immune system, with maintenance of native conformation and biological activity. Protection of antigens from degradation following oral delivery. Probable controlled or slow release properties.

APPEARANCE: White, fine grained suspension or precipitate. May be lyophilized to a white powder.

MOLECULAR WEIGHT: Macromolecular structure of varying size depending on antigen and lipid content.

RECOMMENDED STORAGE: Protein cochleates are stable for at least six months at 4 °C. Alternatively, they may be lyophilized and stored at room temperature for six months as a powder, and reconstituted with liquid prior to administration. Storage for longer time periods and higher temperatures has not yet been assessed.

CHEMICAL/PHYSICAL PROPERTIES: Protein cochleates are formed at or near neutral pH. Their stability to extremes of pH has not been characterized, but they are capable of protecting associated antigens when given orally. This is probably due to their unique rolled-up solid precipitate structure which prevents the exposure of antigens within the interior of the spiral to the external milieu.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: The phospholipids used in the preparation of protein cochleates have been used in humans for vaccines and drug delivery with no significant negative side effects. Protein cochleates have been given to hundreds of mice by various routes including oral, intramuscular, and intranasal, with no negative local or systemic effects noted.

ADJUVANT PROPERTIES: Protein cochleates stimulate strong mucosal and systemic antibody, proliferative and cytotoxic responses to associated antigens. They also afford protection from degradation following oral delivery and probable slow release properties.

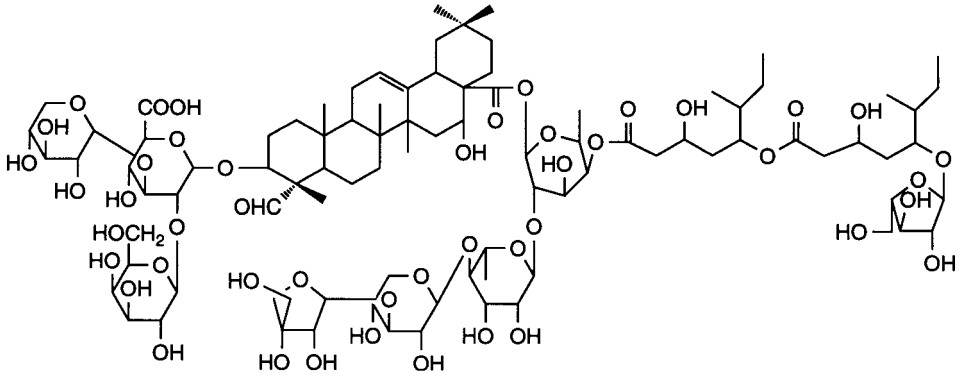
- Gould-Fogerite, S. and Mannino, R. J., 1992, Targeted fusogenic liposomes: Functional reconstitution of membrane proteins into large unilamellar vesicles via protein-cochleate intermediates In: Liposome Technology: 2nd Edition (G. Gregoriadis, Ed.) CRC Press, Inc., Boca Raton, FL Vol. III, Chapter 17: pp. 262-275.
- Gould-Fogerite, *et al*., 1994, Lipid matrix-based subunit vaccines: a structure-function approach to oral and parenteral immunization. *AIDS Res Hum Retroviruses* 10(Suppl 2):S99-103.
- Mannino, R. J. and Gould-Fogerite, S., 1995, Lipid matrix-based vaccines for mucosal and systemic immunization, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Dr. Susan Gould-Fogerite and Dr. Raphael J. Mannino, UMDNJ, New Jersey Medical School, Dept. of Laboratory Medicine and Pathology, Newark, NJ 07103-2714. Ph: 201-982-7836; Fax: 201-982-7293.

COMPONENT/ADJUVANT NAME: QS-21

OTHER NAME(S): Stimulon™ QS-21 Adjuvant.

STRUCTURE:



SOURCE: Natural product of the bark of the *Quillaja saponaria* Molina tree (species native to Chile and Argentina). Extracted from the bark by aqueous extraction. Purified by normal phase and reverse phase chromatography.

- Kensil, C. R. *et al*., 1991, Separation and characterization of saponins with adjuvant activity from Quillaja saponaria Molina cortex. *J. Immunol*., 146:431-437.

USES: Used in vaccine formulations as a primary adjuvant component for enhancement of both humoral and cell-mediated immunity. Water soluble. No emulsification required. Can be used alone or combined with aluminum hydroxide adjuvant.

APPEARANCE: Solid: white odorless powder. Aqueous solution: clear, colorless solution.

MOLECULAR WEIGHT: Parent: 1990, sodium salt: 2012.

RECOMMENDED STORAGE: Store solid QS-21 under low humidity conditions at -20° C. Protect from light. Optimum storage conditions are under evaluation. No apparent degradation under low humidity conditions after storage at 25° C for three years. Aqueous solutions are optimally stable between pH 5 to 7 and in micellar form. Solutions of QS-21 in 0.5 mg/mL solution may be stored in this pH range at 5° C for two or three years. QS-21 is less stable at lower concentrations; HPLC analysis is recommended for analysis of any new vaccine formulation. Protect from light. In aqueous solution, the fatty acid ester bond migrates between the 3 and 4 position on fucose, with the ester at the 4 position being favored. Both forms are active as adjuvants. Primary degradation reaction is alkaline hydrolysis of the fatty acid ester bond at the 3 or 4 position on fucose. Due to alkaline-catalyzed degradation reaction, sterilization should be carried out by membrane filtration instead of autoclaving.

- Cleland, J.L., *et al*., 1996, isomerization and formulation stability of the vaccine adjuvant QS-21, *J. Pharm. Sci*., 85:22-28.

CHEMICAL/PHYSICAL PROPERTIES: Amphiphilic molecule with good water solubility above pKa of carboxyl group (solubility approximately 2 mg/mL to pH 4,15 mg/mL at pH 5,28 mg/mL at pH 6, and 32 mg/mL at pH 7 in buffered saline solutions). Forms micelles in aqueous solution (cmc approximately 50 µg/ml, in phosphate-buffered saline, pH 7.0).

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: QS-21 has been entered in a Phase III trial of a therapeutic melanoma vaccine at 100 µg QS-21 per dose. QS-21 has been evaluated in Phase I and II trials of 31 different vaccines over a QS-21 dose range of 25 to 100 µg. At present, over 1500 individuals have received QS-21 adjuvanted vaccines.

- Livingston, P. 0. *et al*., 1994, Phase I trial of immunological adjuvant QS-21 with a G_{M2} ganglioside-KLH conjugate vaccine in patients with malignant melanoma. Vaccine, 12:1275-80.

ADJUVANT PROPERTIES: Shown to stimulate humoral immune responses in mice, including antigen-specific IgG1, IgG2b and IgG2a titers. Most QS-21 formulations in mice have been administered by the subcutaneous or intramuscular route, but intranasal and oral administration have also been shown to be effective. Augments production of IgG responses to ganglioside antigen in melanoma vaccine in human Phase I clinical trials. Augments protective benefit of a recombinant malaria vaccine in human Phase I clinical trials. Shown also to stimulate CTL responses in mice.

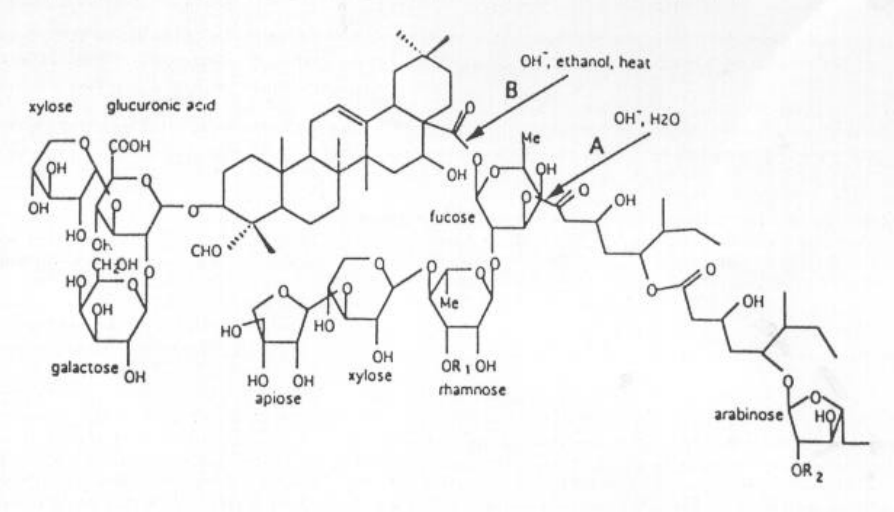
- Kensil, C. R. *et al*., 1993, The use of Stimulon adjuvant to boost vaccine response. *Vaccine Research*, 2:273-281.
- Newman, M., J. *et al*., 1992, Saponin adjuvant induction of ovalburnin specific CD8+ cytotoxic T--lymphocyte responses. *J. Immunol*., 138:2357-2362.
- Helling, F. *et al*., 1995, GM2-KLH conjugate vaccine: Increased immunogenicity in melanoma patients after administration with immunological adjuvant QS-21, *Cancer Res*., 55:2783-2788.
- Stoute, J.A. *et al*., 1997, A preliminary evaluation of a recombinant circumsporozoite protein vaccine against Plasmodium falciparium malaria. *New England J. of Med*., 336:86-91.
- Kensil, C. *et al*., 1995, Structural and immunological characterization of the vaccine adjuvant QS-21, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York., pp. 525-541.

CONTACT(S): Dr. Charlotte Kensil, Aquila Biopharmaceuticals, Inc. Ph: 508-797-5777; Fax: 508-797-4014.

COMPONENT/ADJUVANT NAME: Quil-A

OTHER NAME(S): Quil-A saponin, *Quillaja* saponin

STRUCTURE: A complex but purified mixture of Quillaja saponins which are glycosides of Quillaic acid and carbohydrates. The Higuchi formula of Quil A is shown below.



SOURCE: Purified extract from the bark of the South American tree *Quillaja saponaria* Molina.

USES: Quil-A is used in veterinary vaccines and for production of ISCOMs.

APPEARANCE: Lyophilized powder. Color is light brownish, almost white.

MOLECULAR WEIGHT: Ranging from approximately 1400-2400 D.

RECOMMENDED STORAGE: Dry storage in the Iyophilized state. Can be stored frozen, refrigerated or at room temperature.

CHEMICALIPHYSICAL PROPERTIES: The mixture contains fractions that bind to cholesterol, are adjuvant active, is hernolytic, and are able to form ISCOMs.

INCOMPATIBILIT'Y: Should not be exposed to alkaline conditions (pH > 8.0).

SAFETY/TOXICITY: Avoid inhalation and eye contact when handling Quil-A. Quil-A is highly irritating to mucosa. Quil-A contains hemolysing saponins. Quil A is not used in human trials because of overt toxicity. It is, however, used extensively in veterinary vaccines.

- G. J. A. Speijers et al, Local reactions of the saponin Quil A and a Quil A containing iscorn measles vaccine after intramuscular injection of rats: A comparison with the effects of DTP-poho vaccine, Fund. Appl. Tox., 10:425-430 (1988).

ADJUVANT PROPERTIES: Quil A is used a part of ISCOMs. as well as with antigen alone. It induces both humoral and a cell-mediated responses.

- Morein, B., 1984, Iscom, a novel structure for antigenic presentation of membrane proteins from enveloped viruses, *Nature* 308:457-460.

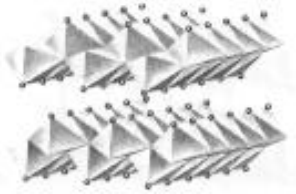
- Dalsgaard, K. *et al*., 1977, Evaluation of the adjuvant "Quil-A" in the vaccination of cattle against foot-and-mouth disease. *Acta Vet. Scand*., 18:349-360.
- Dalsgaard, K., 1984, Assessment of the dose of the immunlogical adjuvant Quil-A in mice and guinea pigs, using sheep red blood cells as model antigen. *Zbl. Vet. Med. B*., 31:718-720.
- Dalsgaard, K. and Jensen, M. H., 1977, The adjuvant activity of "Quil-A" in trivalent vaccination of cattle and guinea pigs against foot-and-mouth disease. *Acta Vet. Scand*., 18:367-373.
- Osterhaus, A. and Rimmelzwaan G. F., 1995, A novel generation of viral vaccines based on the ISCOM matrix, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
  Campbell, J.B., 1995, Saponins, in: The Theory and Practical Application of Adjuvants, Stewart-Tull (Ed.), Wiley and Sons.

CONTACT(S): There are several suppliers of Quil A. This monograph prepared by: E. B. Lindblad, Superfos Biosector, DK-2950 Vedbaek, Denmark, Ph: 45 47 38 47 00; Fax: 47 38 46 56. Also: Al Reisch, Sargeant, Inc., Clifton, NJ 07012, Ph: 201-472-9111; Fax: 201-472-5636. Also: Accurate Chemical & Scientific Corp. Westbury, NY 11590, Ph: 800-645-6264; Fax: 516-997-4948.

COMPONENT/ADJUVANT NAME: Rehydragel HPA

OTHER NAME(S): High Protein Adsorbency Aluminum Hydroxide Gel; alum

STRUCTURE: Crystalline aluminum oxyhydroxide AlOOH, known minerologically as boehmite. the structure consists of corrugated sheets of aluminum octahedra.



SOURCE: Synthetic oxyhydroxide of aluminum (aluminum hydroxide) prepared by acid-base precipitation.

USES: Primary adjuvant in parenteral vaccine formulations. Does not generally induce cell mediated immunity.

APPEARANCE: Translucent, thixotropic, colloidal aqueous gel supplied sterile.

MOLECULAR WEIGHT: 60 (empirical formula).

RECOMMENDED STORAGE: Stable at room temperature for indefinite period. Freezing should be avoided.

CHEMICAL/PHYSICAL PROPERTIES: Contains 2% equivalent Al_2O_3 or 3% equivalent $Al(OH)_3$. Primary particles have a rod-like morphology and a high surface area. The isoelectric point is 11. Its high surface area gives it a high adsorptive capacity for antigens. Typical pH: 5.8 to 6.8. Insoluble in water between pH 4 to 8, and poorly soluble in solutions containing citrate ion. Average particle size: submicron. Thixotropic/pumpable suspension.

INCOMPATIBILITY: Do not freeze, otherwise chemically inert and stable.

SAFETY/TOXICITY: Aluminum compounds (aluminum hydroxide, aluminum phosphate, alum) are currently the only vaccine adjuvants used in US-licensed vaccines. They can induce granulomas at the inoculation site. Supplied pyrogen free.

- Ganrot, P. 0., 1986, Metabolism and possible health effects of aluminum. *Environ. Health Persp*., 65:363-441.

ADJUVANT PROPERTIES: Protein binding capacity: 2.5 mg BSA/mg Al_2O_3 minimum. The surface area, surface charge, and morphology of the aluminum hydroxide are major factors in its adjuvant characteristics. The use of aluminum adjuvants are accompanied by stimulation of IL-4 and stimulation of the T-helper-2 subsets in mice, with enhanced IgG1 and IgE production.

- Shirodkar, S. *et al*. Aluminum compounds used as adjuvant in vaccines, *Pharm. Res*., 7:1282-88.
- Aprile, M. A. and Wardlaw, A. C., 1966, Aluminum compounds as adjuvants for vaccines and toxoids in man. *Can. J. Publ. Hlth*., 57:343-354.
- Gupta, R., 1995, Adjuvant properties of aluminum and calcium compounds. in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
- Seeber, S. *et al*., 1991, Predicting the adsorption of proteins by aluminum-containing adjuvants. Vaccine 9:201-203.

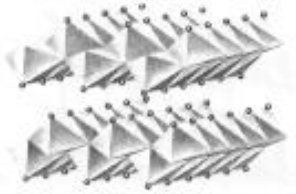
- Seeber, S. J., *et al*, 1991, Solubilization of aluminum-containing adjuvants by constituents of interstitial fluid. J. Parenteral Sci. Tech., 45: 156-159.
- Hem, S. and White, J. L., 1995, Structure and properties of aluminum containing adjuvants. in: Vaccine Design- M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Philip B. Klepak, Reheis Inc., Berkeley Heights, NJ 07922, Ph: 908-464-1500; Fax: 908-464-7726. Also: Stanley Hem, Purdue University, West Lafayette, IN 47907-1336, Ph: 317-494-1451; Fax: 317-496-1484.

COMPONENT/ADJUVANT NAME: Rehydragel LV

OTHER NAME(S): low viscosity alluminurn hydroxide gel; alum

STRUCTURE: Crystalline aluminum oxyhydroxide AlOOH, known minerologically as boehmite. the structure consists of corrugated sheets of aluminum octahedra.



SOURCE: Synthetic oxyhydroxide of aluminum (aluminum hydroxide) prepared by acid-base precipitation.

USES: Primary adjuvant in parenteral vaccine formulations. Does not generally induce cell mediated immunity.

APPEARANCE: White, fluid aqueous suspension supplied sterile.

MOLECULAR WEIGHT: 60 (empirical formula)

RECOMMENDED STORAGE: Stable at room temperature for indefinite period. Freezing should be avoided.

CHEMICAL/PHYSICAL PROPERTIES: Contains 2% equivalent A1203 or 3% equivalent AI(OH)3. Primary particles have a rod-like or fibril morphology, but are larger than Rehydragel HPA. Surface area and antigen absorptive capacity diminished compared with Rehydragel HPA. Typical pH is 5.8 to 6.8 Insoluble in water between pH 4 to 8, and poorly soluble in solutions containing citrate ion. Average particle size: 1 micron. Has a low viscosity and is pumpable.

INCOMPATIBILITY: Do not freeze, otherwise chemically inert and stable.

SAFETY/TOXICITY: Aluminum compounds (aluminum hydroxide, aluminum phosphate, alum) are currently the only vaccine adjuvants used in US-licensed vaccines They can induce granulomas at the inoculation site. Supplied pyrogen free.

- Ganrot, P. 0., 1986, Metabolism and possible health effects of aluminum. *Environ. Health Persp*., 65:363-441.

ADJUVANT PROPERTIES: Protein binding capacity: 1.5 mg BSA/mg equivalent Al_2O_3 minimum. The surface area, surface charge, and morphology are major factors in its adjuvant characteristics. The use of aluminum adjuvants are accompanied by stimulation of IL-4 and stimulation of the T-helper-2 subsets in mice, with enhanced IgG1 and IgE production.

- Aprile, M. A. and Wardlaw, A. C., 1966, Aluminum compounds as adjuvants for vaccines and toxoids in man. *Can. J. Publ. HIth*., 57:343-354.
- Gupta, R., 1995, Adjuvant properties of aluminum and calcium compounds. in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.
- Seeber, S. *et al*., 1991, Predicting the adsorption of proteins by aluminum-containing adjuvants. *Vaccine* 9:201-203.
- Seeber, S. J., *et al*, 1991, Solubilization of aluminum-containing adjuvants by constituents of interstitial fluid. J. Parenteral Sci. Tech., 45: 156-159.

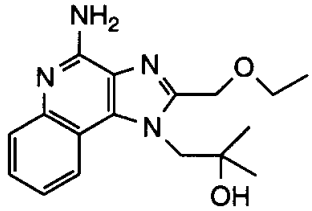
- Hem, S. and White, J. L., 1994, Structure and properties of aluminum containing adjuvants. in: Vaccine Design- M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Philip B. Klepak, Reheis Inc., Berkeley Heights, NJ 07922, Ph: 908-464-1500; Fax: 908-464-7726. Also: Stanley Hem, Purdue University, West Lafayette, IN 47907-1336, Ph: 317-494-1451; Fax: 317-496-1484.

COMPONENT/ADJUVANT NAME: S-28463

OTHER NAME(S): 4-Amino-otec,-dimethyl-2-ethoxymethyl-l*H*-imidazo[4,5-c]quinoline-1-ethanol

STRUCTURE:



SOURCE: Chemical synthesis. International Publication 92/15582.

USES: Included in adjuvant formulations as a primary adjuvant component.

APPEARANCE: White, fine crystalline solid.

MOLECULAR WEIGHT: 314.39 free base, 350.85 hydrochloride salt.

RECOMMENDED STORAGE: Solid is stable at room temperature. Shelf life is acceptable.

CHEMICAL/PHYSICAL PROPERTIES: Some water solubility as the free base. The hydrochloride salt is soluble in water at concentrations at least to 10 mg/mL.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: In preclinical animal safety evaluation studies.

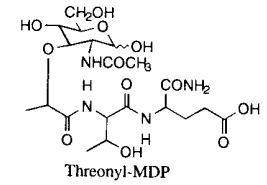
ADJUVANT PROPERTIES: Addition of S-28463 induces both humoral and cell-mediated immunity via induction of cytokines from monocytes and macrophages. Unpublished results indicate S-28463 is about 100-fold more potent than imiquimod in antiviral models and in cytokine induction from monocytes and macrophages.

CONTACT: R. C. Hanson, Business Development, 3M Pharmaceuticals, St. Paul, MN 55144, Ph: 612-737-3137; Fax: 612-737-4556.

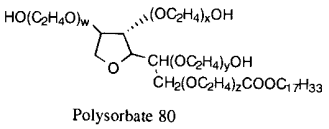
COMPONENT/ADJUVANT NAME: SAF-1

OTHER NAME(S): SAF-m; Syntex Adjuvant Formulation

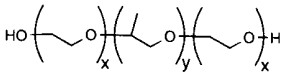
STRUCTURE: Composed of threonyl-MDP (0.05-1%) in an emulsion vehicle [5% squalane, 2.5% Pluronic® L121, 0.2% Polysorbate 80 and phosphate buffered saline (pH 7.4)].



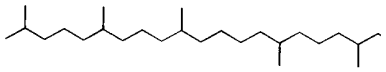
Threonyl-MDP



Polysorbate 80



Pluronic L121


Squalane

SOURCE: See individual components.

APPEARANCE: White, fluid, oil-in-water emulsion.

MOLECULAR WEIGHT: n/a (see individual components).

RECOMMENDED STORAGE: $\geq$30º C.

CHEMICAL/PHYSICAL PROPERTIES: Particle size depends on the manufacturing method used. If the emulsion is manufactured using a microfluidizer, then the mean particle size is -160 ran.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: At therapeutic doses, no safety concern is anticipated. Dose is indication dependent, but a typical volume of injection is 1 mL.

ADJUVANT PROPERTIES: Antigens become arranged on the surface of the emulsion droplets partly because of their amphipathic nature, and partly because of hydrogen bonding with poloxamer 401. The emulsion droplets also activate complement, as demonstrated by consumption of C3 and production of C3b; the latter, on the surface of droplets, targets them to antigen-presenting cells (follicular dendritic cells and interdigitating cells) in lymph nodes of the drainage chain and possibly in more distant lymphoid tissues. In this way the emulsion facilitates the presentation of antigens to responding lymphocytes. See threonyl-MDP monograph.

- Allison, A. C. and Byars, N. E., 1987, Syntex adjuvant formulation. *Res. in Immunol.*, 143:519-525 (1992).
- Byars, N. E. and Allison, A. C. Adjuvant formulation for use in vaccine to elicit both cell mediated and humoral immunity. *Vaccine* 5:223-228.
- Allison, A. C. and Byars, N. E., 1986, An adjuvant formulation that selectively elicits the formation of antibodies of protective isotypes and of cell-mediated immunity. *J. Immunol. Meth.*, 95:157-168.
- Lidgate, D. M. and Byars, N., 1995, Development of an emulsion based muramyldipeptide adjuvant formulation for vaccines, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Gary Ott, Chiron Corporation, Emeryville, CA, Ph: 510-923-2964; Fax: 510-923-4265.

COMPONENT/ADJUVANT NAME: Sclavo peptide

OTHER NAME(S): IL-1β 163-171 peptide

STRUCTURE: VQGEESNDK •HCl

SOURCE: From human IL-1β amino acid sequence. Obtained by solid phase synthesis, purified by HPLC and ion exchanged to the HCI salt.

USES: Primary adjuvant. Active either when administered separately from antigen, or admixed with antigen, or physically linked to antigen. Routes of administration: i.v., i.p., s.c., p.o. Marked adjuvant activity is also observed upon injection of the coding sequence in plasmid DNA vaccines.

APPEARANCE: White, odorless powder.

MOLECULAR WEIGHT: 1 kD

RECOMMENDED STORAGE: Stored lyophilized peptide dry at -20º C. Stable also at room temperature. The concentrated solution can be stored in siliconized glass at 4º C for at least 2-3 months. Do not freeze.

CHEMICAL/PHYSICAL PROPERTIES: Good solubility in water. Very acidic. Adjust pH to neutrality before use.

INCOMPATIBILITY: Avoid peptidases.

SAFETY/TOXICITY: No toxicity in mice when given i.v. as a bolus up to 100 mg/kg.

ADJUVANT PROPERTIES: It enhances immune response to T-dependent and T-independent antigens. Active also in increasing secondary responses. Active as adjuvant for a tumor vaccine. Antitumor activity through recruitment of host immune response.

- Nencioni, L. *et al*., 1987, In vivo immunostimulating activity of the 163-171 peptide of human IL-1β. *J. Immunol*., 139:800-804.
- Boraschi, D. *et al*., 1988, In vivo stimulation and restoration of the immune response by the noninflammatory fragment 163-171 of human IL-1β. *J. Exp. Med*., 168:675-686.
- McCune, C. S. and Marquis, D. M., 1990, Interleukin-1 as an adjuvant for active specific immunotherapy in a murine tumor model. *Cancer Res*., 50:1212-1215.
- Rao, K. V. S. and Nayak, A. R., 1990, Enhanced immunogenicity of a sequence derived from hepatitis B virus surface antigen in a composite peptide which includes the immunostimulatory region from human interleukin-1. *Proc. Natl. Acad. Sci. USA*, 87:5519-5522.
- Beckers, W. *et al*., 1993, Increasing the immunogenicity of protein antigens through the genetic insertion of VQGEESNDK sequence of human IL-1β into their sequence. *J. Immunol*., 151:1757-1764.
- Hakim, I. *et al*., 1996, A nine amino acid peptide from IL-1β augments antitumor immune responses induced by protein and DNA vaccines, *J. Immunol*. 157:5503-11.

CONTACT(S): Dr. D. Boraschi, Dompè Research Center, L'Aquila, Italy, Ph: 39-862-338324; Fax: 39-862-338219. Also Dr. A. Tagliabue, University of Bologna, Italy. Ph: 39-335-6154151; Fax 39-51-354224.

COMPONENT/ADJUVANT NAME: Sendai Proteoliposomes, Sendai-containing Lipid Matrices

OTHER NAME(S): Sendai glycoprotein-containing vesicles; fusogenic proteoliposomes; FPLs; Sendai lipid matrix-based vaccines.

STRUCTURE: Sendai proteoliposomes: The glycoproteins of Sendai virus (parainfluenza type 1) are integrated in the lipid bilayers, of large, mainly unilamellar, liposomes. Native conformation and biological activities of receptor binding and membrane fusion are maintained. Other proteins containing hydrophobic regions or lipid anchored proteins or peptides may be encapsulated in the hpid bilayer. Water soluble proteins or other materials may be encapsulated in the aqueous interior of the vesicles.
Sendai-containing lipid matrices: Some peptides which are amphipathic (i.e., possess both hydrophilic and hydrophobic regions) have the ability to collapse lipid bilayers. When these peptides are encapsulated by adding EDTA to Sendai protein cochleates, lipid aggregates, rather than liposomes (with a continuous lipid bilayer encapsulating an internal aqueous space) are produced. Polymorphic lipid aggregates also form when plain lipid cochleates are converted by EDTA in the presence of high concentrations of these amphipathic peptides.

SOURCE: Prepared from Sendai protein cochleates by chelation of Ca^{++} with EDTA. See Protein cochleates for lipids and antigens used and sources. Material encapsulated includes chemically synthesized peptides, isolated and recombinant proteins, whole fixed viruses, small molecule drugs and DNA.

USES: Sendai proteoliposomes produced by these methods are highly effective immunogens in mice, rabbits and monkeys. This includes the ability to stimulate strong T helper and CD8+ cytotoxic T cell responses (CTL) to lipid bilayer-integrated glycoproteins as well as encapsulated peptides, proteins and whole formalin-fixed viruses. These vesicles also act as effective delivery vehicles for drugs and proteins. They were used to achieve the first stable gene transfer in animals using a liposome based system. These abilities probably arise from their membrane attachment and fusion activity which facilitates introduction into the cytoplasm and access to an MHC Class I presentation pathway.

APPEARANCE: Opalescent suspension in aqueous isotonic buffer.

MOLECULAR WEIGHT: Macromolecular structure of varying size depending on antigen and lipid content.

RECOMMENDED STORAGE: Phospholipids used as raw materials are stored in chloroform at 20° C. under nitrogen. Proteoliposomes should be stored at 4° C in isotonic buffer. They are generally used within a few days of preparation. Long term stability has not been assessed.

CHEMICAL/PHYSICAL PROPERTIES: Proteoliposomes are stable in aqueous isotonic buffers. They are solubilized by detergents or organic solvents in sufficient quantities.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: The phospholipids used in the preparation of proteoliposomes have been used in humans for vaccines and drug delivery with no significant negative side effects. Proteoliposomes have been given to hundreds of mice, by intraperitoneal and intramuscular immunization, and many rabbits and sixteen monkeys by intramuscular immunization with no negative local or systemic effects noted.

ADJUVANT PROPERTIES: Proteoliposomes stimulate strong antibody and proliferative responses to associated antigens. They are particularly powerful inducers of cytotoxic T lymphocytes. Antigens can be associated with the lipid bilayer or encapsulated within the aqueous interior.

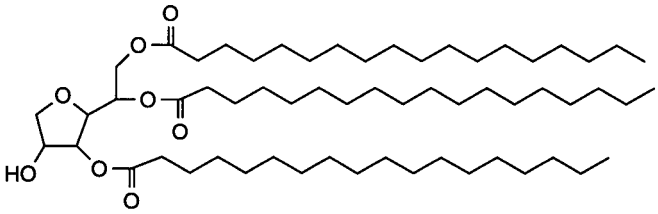
- Gould-Fogerite, S. *et al.*, 1989, Chimerasome-mediated gene transfer in vitro and in vivo. Gene, 84:429438.
- Gould-Fogerite, S. and Mannino, R. J., 1992, Targeted fusogenic liposomes: Functional reconstitution of membrane proteins into large unilamellar vesicles via protein-cochleate intermediates In: Liposome Technology: 2nd Edition ,G. Gregoriadis, (Ed.) CRC Press, Inc., Boca Raton, FL Vol. III, Chapter 17: pp. 262-275.
- Miller, M.D., *et al.*, 1992, Vaccination of Rhesus Monkeys with synthetic peptide in a fusogenic proteoliposome elicits simian immunodeficiency virus-specific $CD8^+$ cytotoxic T lymphocytes. *J. Exp. Med*. 176:1739-1744.
- Mannino, R. J. and Gould-Fogerite, S., 1995, Lipid matrix-based vaccines for mucosal and systemic immunization, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Dr. Susan Gould-Fogerite and Dr. Raphael J. Mannino, UMDNJ, New Jersey Medical School, Dept. of Laboratory Medicine and Pathology, Newark, NJ 07103-2714. Ph: 201-982-7836; Fax: 201-982-7293.

COMPONENTIADJUVANT NAME: Span 85

OTHER NAME(S): Arlacel 85, sorbitan trioleate

STRUCTURE: Spans are partial esters of common fatty acids (lauric, palmitic, stearic and oleic) and hexitol anhydrides (hexitans and hexides), derived from sorbitol. An example structure is shown below.



SOURCE: Synthetic.

USES: Used as an emulsification agent in MF59 adjuvant formulation.

APPEARANCE: Viscous yellow liquid.

MOLECULAR WEIGHT: Most spans are actually mixtures with one particular span predominating.

RECOMMENDED STORAGE: Store in a cool dry place.

CHEMICAUPHYSICAL PROPERTIES: Span products tend to be oil-soluble. Span 85 is insoluble in water but can be dispersed with a hydrophilic surfactant. Density 0.956.

INCOMPATIBILITY: Strong oxidizing agents.

SAFETY/TOXICITY: Vapor or mist is irritating to mucous membranes. Causes skin irritation.

ADJUVANT PROPERTIES: None described for the compound itself. See MF-59.

- Van Nest, G. A. *et al*., 1995, MF59: Design and evaluation of a safe and potent adjuvant for human vaccines, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Several suppliers offer Span 85. Sigma Chemical Company, Ph: 800-325-3010. For vaccine formulation use: Gary Ott, Chiron Corporation, Emeryville, CA, Ph: 510-923-2964; Fax: 510-923-4265.

COMPONENT/ADJUVANT NAME: Specol

STRUCTURE(S): Marcol 52 (mineral oil, paraffins, and cycloparaffins, chain length 13-22 C atoms)
Span 85 (emulsifier, sorbitan trioleate)
Tween 85 (emulsifier, polyoxyethylene-20-trioleate)

SOURCE: Ingredients are commercially available and all are individually FDA approved for veterinary use. Mineral oil and emulsifiers (Span and Tween) are thoroughly mixed 9:1 (v/v) and can be stored at 4° C for prolonged periods of time (several years).

USES: Specol can be obtained from ID-DLO in Lelystad and is a primary adjuvant (only antigen needed). The adjuvant mixture of mineral oil and emulsifiers is mixed with the water phase (physiological saline) containing the immunogen (water: oil = 0.44) and emulsified. A stable emulsion is obtained when the second of two drops, deposited on the surface of a water-containing tube, continues to float intact. When a stable (sterile) emulsion is obtained this can be stored for up to 1 year at 4-16° C or 3 months at 37° C (dependent on antigen). It functions as a depot (slow release of antigen) and a polyclonal activator (independent of presence of antigen) for cells of the immune system (cytokine release).

APPEARANCE: Specol is a clear oily fluid. The water-in-oil (w/o) emulsion resulting from mixing Specol with immunogen/water is white and gel-like.

MOLECULAR WEIGHT: Not applicable.

RECOMMENDED STORAGE: 4° C in low-oxygen conditions (e.g., in completely filled bottles or under NA As stated below, both Specol and the emulsion are relatively insensitive to temperature changes.

CHEMICAL/PHYSICAL PROPERTIES: The water-in-oil emulsion (Specol emulsified with immunogen in water) is resistant to temperature shifts (4-37° C). It has a low conductivity (indicative of proper separation of oil and water) of <0.6 siemens and a low viscosity (enabling easy application by injection) of 70-100 mPa/s (both measured at 20° C).

INCOMPATIBILITY: The Specol-water/immunogen emulsion is not compatible with natural rubber and is probably incompatible with most organic solvents as is common for water-in-oil emulsions.

SAFETY: No known use in humans, registered for veterinary use by itself (nonspecific stimulation of immune system, e.g., in weanlings) or in combination with vaccines.

ADJUVANT PROPERTIES: The adjuvant properties of Specol, which are comparable to CFA, in rodents are reviewed in:

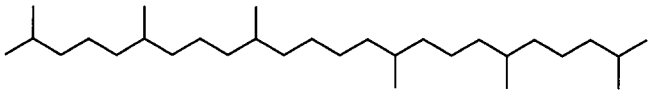
- Boersma, W. J. A., *et al*., 1992, Adjuvant properties of stable water-in-oil emulsions, evaluation of the experience with Specol. 44th Forum in Immunology, *Res. Immunol. 143:503-512.*
- Bokout, B. *A., et al.*, 1981, *A* selected water in oil emulsion: Composition and usefulness as an immunological adjuvant, *Vet. Immunol. Immunopathol: 2:491-500.*

CONTACT(S): Source: Dr. B. Bokout, Institute of Animal Science and Health, POB 65, 8200 AB, The Netherlands, Ph: + 31 3200 73432; Fax: + 31 3200 73473; email: l3.A.l3okbout@CDI.AGR0.NL. Rodent studies: Prof. Dr. E. Claassen, TNO-Prevention and Health, Fax + 31 71 181276.

COMPONENT/ADJUVANT NAME: Squalane

OTHER NAME(S): Spinacane;Robane®;2,6,10,15,19,23-hexamethyltetracosane

STRUCTURE:



SOURCE: Obtained by the total hydrogenation of the triterpene Squalene, a component of shark Ever oil and some vegetable oils.

USES: Component of Antigen Formulation (AF) and Syntex Adjuvant Formulation (SAF), present in final concentration of 5% w/v with antigen. Constitutes the oil component of the emulsion. A metabolizable oil, used in cosmetics, topicals and as a vehicle for lipophilic drugs.

APPEARANCE: Clear oil.

MOLECULAR WEIGHT: 422.83.

RECOMMENDED STORAGE: Airtight container at room temperature.

CHEMICAUPHYSICAL PROPERTIES: Stable to air and oxygen. Readily soluble in organic solvents, slightly soluble in alcohol. Specific gravity 0.807-0.810 at 20° C.

INCOMPATIBILITY: None found.

SAFETY:

- Christian, M. S., 1982, Final report on the safety assessment of squalane and squalene. *J. Amer. Coll. Toxicol.*, 1:37-56.

ADJUVANT PROPERTIES: Squalane itself is not an adjuvant. See monographs on SAF-1 and AF.

- Lidgate, D. M. and Byars, N., 1995, Development of an emulsion based muramyl dipeptide adjuvant formulation for vaccines, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Supplied by several companies. For adjuvant use contact: Thomas Ryskamp, IDEC Pharmaceuticals Corporation, San Diego, CA 92121, Ph: 619-550-8500, Fax: 619-550-8750, Internet: tryskamp@idec.com.

COMPONENT/ADJUVANT NAME: Squalene

OTHER NAME(S): Spinacene; Supraene; 2,6,10,15,19, 23-hexamethyl-2,6,10,14,18,22 tetracosahexaene

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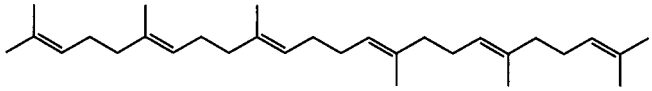


SOURCE: Found in shark liver oil and some vegetable oils. Intermediate in the biosynthesis of cholesterol.

USES: Bactericide, intermediate in the manufacturing of pharmaceuticals, component of MF59 emulsion formulation, constitutes the oil component of the emulsion.

APPEARANCE: Clear oil, colorless. Faint, agreeable odor.

MOLECULAR WEIGHT: 410.7

RECOMMENDED STORAGE: Store in a cool place.

CHEMICAL/PHYSICAL PROPERTIES: A metabolizable oil. Practically insoluble in water, highly soluble in organic solvents, may become viscous upon absorbing oxygen. Specific gravity 0.858. Bp 285° C/25mm.

INCOMPATIBILITY: Avoid oxidizers.

SAFETY: May be harmful by inhalation, ingestion or percutaneous adsorption. Oral LD50 5 g/kg, IV LD50 1.8 g/kg.

- Christian, M. S., 1982, Final report on the safety assessment of squalane and squalene. *J. Amer. Coll. Toxicol.*, 1:37-56.

ADJUVANT PROPERTIES: Squalene itself is not an adjuvant. See monograph on MF59.

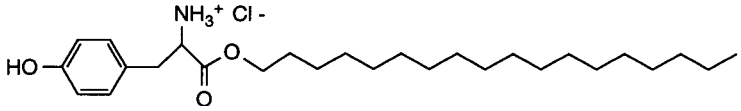
- Sanchez-Pestador, L., *et al*., 1988, The effect of adjuvants on the efficacy of a recombinant herpes simplex glycoprotein vaccine, J. Immunol., 141:1720-1727.
- Van Nest, G. A., *et al*, 1992, Advanced adjuvant formulations for use with recombinant subunit vaccines in: Vaccines 92, Ed by F. Brown, RM. Chanock, H. S. Ginsberg and R. A. Lerner, Cold Spring Harbor Press, Plainview, NY, pg 57.
- Van Nest, G. A. *et al*., 1995, MF59: Design and evaluation of a safe and potent adjuvant for human vaccines, in: Vaccine Design M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Supplied by several companies. For example, Sigma Chemical Company Ph: 800-3253010. For vaccine formulation use: Gary Ott, Chiron Corporation, Emeryville, CA, Ph: 510-923-2964; Fax: 510-923-4265.

COMPONENT/ADJUVANT NAME: Stearyl Tyrosine

OTHER NAMEM: Octadecyl tyrosine hydrochloride

STRUCTURE:



SOURCE: Chemical synthesis from tyrosine and stearyl alcohol (octadecanol).

- Penney, C.L. *et al*., 1985, A simple method for the synthesis of long-chain alkyl esters of amino acids, *J. Org. Chem*., 50:1457-59.

USES: Primary vaccine adjuvant with minimal immunostimulatory properties. Some use in allergy desensitization therapy. Biocide.

APPEARANCE: White, amorphous free-flowing, odorless powder.

MOLECULAR WEIGHT: 470.14 (hydrochloride salt)

RECOMMENDED STORAGE: Store solid at room temperature. Aqueous suspensions may be stored at pH 4.0-7.5 at 4º C for several years.

CHEMICAL/PHYSICAL PROPERTIES: Sharp melting point (171-30C). Insoluble (<0.01%) at neutral and alkaline pH; soluble at low pH in hot mineral acid.

INCOMPATIBILITY: Incompatible with strong base.

SAFETY/TOXICITY: Non-toxic up to 2500 mg/kg in many animals, including primates. Nonpyrogenic. No adjuvant arthritis (rats). No damage at site of injection (cats). Biodegradable.

ADJUVANT PROPERTIES: "Organic equivalent" of aluminum hydroxide, with likely carrier depot effect; adjuvanticity similar to aluminum hydroxide with bacterial vaccines; superior to aluminum hydroxide with viral vaccines. Favorable isotype distribution. Biocompatible.

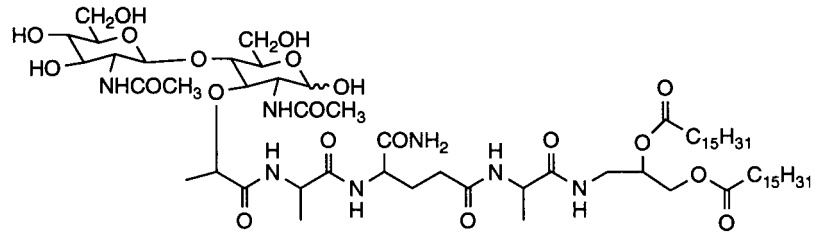
- Penney, C.L. *et al*., 1993, Further studies on the adjuvanticity of stearyl tyrosine and ester analogues, *Vaccine*, 11: 1129-34.
- Penney, C. L. *et al*., 1994, Further studies on the adjuvanticity of stearyl tyrosine and amide analogs. *Vaccine*, 12:629-632.
- Penney, C., 1995, Stearyl tyrosine: An organic equivalent of aluminum immunoadjuvant, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Dr. Christopher L. Penney, Biochem. Therapeutic, Inc., Laval, Que., Canada H7V-4A7, Ph: 514-978-7811; Fax: 514-978-7777.

COMPONENT/ADJUVANT NAME: Theramide™

OTHER NAME(S): *N*-acetylglucosaminyl-*N*-acetylinuramyl-L-Ala-D-isoGlu-L-Ala-dipalmitoxy propylamide (DTP-DPP)

STRUCTURE:



SOURCE: Synthetic.

USES: The drug compound is a potent macrophage activator and adjuvant. It induces IL-6, IL-12, TNF, IFN-γ, and relatively lessor quantities of IL-10. The compound preferentially induces cellular immunity. When reconstituted, it spontaneously forms liposomes in which lipopeptides may be incorporated.

APPEARANCE: White, odorless powder.

MOLECULAR WEIGHT: 1315.84

RECOMMENDED STORAGE: Stable as a lyophilized powder or in solution at room temperature for five years in saline or PBS at pH 7.4.

CHEMICALIPHYSICAL PROPERTIES: Amphoteric molecule soluble in water, phosphate buffered saline, chloroform:methanol (7:3), and tert-butanol.

INCOMPATIBILITY: Avoid strong acids or bases.

SAFETY/TOXICITY: Human Phase I clinical trials at 200ug/m2 to 1000 μg/m^2 i.v. weekly have been initiated.

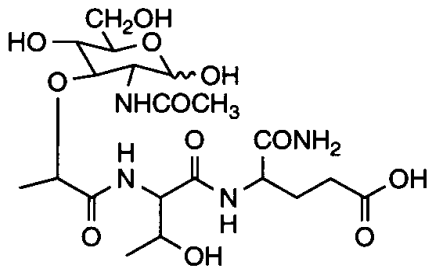
ADJUVANT PROPERTIES: The compound augments both cellular and humoral immunity and is active in murine models of CMV.

CONTACT: Gerald J. Vosika, M.D., ImmunoTherapeutics, Inc., South, Fargo, ND 58104. Ph: 701232-9575; Fax: 701-237-9275.

COMPONENT/ADJUVANT NAME: Threonyl-MDP

OTHER NAME(S): Termurtide™; [thr[1]]-MDP; *N*-acetyl muramyl-L-threonyl-D-isoglutamine

STRUCTURE:



SOURCE: Synthetic.

- G. J. Jones, *et al*, Novel immunological adjuvant compounds and methods of preparation thereof. Syntex, U.S. A., U.S. Patent # 4,082,735.

USES: Threonyl-MDP is included in adjuvant formulations as a primary adjuvant component.

APPEARANCE: White to off-white, odorless powder.

MOLECULAR WEIGHT: 522.5

RECOMMENDED STORAGE: The powdered drug substance should be stored dessicated at or below 25º C. For optimal stability, solutions (0.5-10 mg/mL) of threonyl-MDP should be formulated between pH 3.5 and 5.5; under this condition, a two-year shelf-life at 25º C can be expected. Solutions of threonyl-MDP formulated in a broader pH range of 1.5 to 7.5 show a two-year shelf-life if stored at 5º C.

- M. F. Powell et al., 1988, Formulation of vaccine adjuvant muramyldipeptides. 2. Thermal reactivity and pH of maximum stability of MDP compounds in aqueous solution. Pharm. Res. 5:528.

CHEMICALIPHYSICAL PROPERTIES: Threonyl-MDP has an aqueous solubility of >600 mg/mL. The pKa of the isoglutamine carboxylic acid is 4.3. The compound is very hygroscopic and found to deliquesce at $\geq$ 68% relative humidity.

INCOMPATIBILITY: Avoid strong bases.

SAFETY/TOXICITY: At therapeutic doses, no safety concern is anticipated. Dose is indication dependent, but a guideline is 0.05-1% (w/w), with an injection volume of -1 mL.

ADJUVANT PROPERTIES: Threonyl-MDP induces the production of a cascade of cytokines, including IL-1α, IL-1β and IL-6. Responding lymphocytes release IL-2 and IFN-γ. The latter increases the production of antibodies of certain isotypes, including IgG2a. in the mouse. This isotype, and the homologous IgG1 in primates, interacts with high affinity Fcγ receptors, so that the antibodies can function efficiently in opsonizing viruses and other infectious agents for uptake by phagocytic cells.

- Allison, A. C. and Byars, N. E. Syntex Adjuvant Formulation. Res. Immunol., 143:519-525 (1992).
- Allison, A. C. and Byars, N. E. An Adjuvant Formulation that Selectively Elicits the Formation of Antibodies of Protective Isotypes and of Cell-Mediated Immunity. J. Immunol. Meth., 95:157-168 (1986).

- Lidgate, D. M. and Byars, N., 1995, Development of an emulsion based muramyl dipeptide adjuvant formulation for vaccines, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): Gary Ott, Chiron Corporation, Emeryville, CA, Ph: 510-923-2964; Fax: 510-923-4265.

COMPONENTIADJUVANT NAME: Ty Particles

OTHER NAME(S): Ty-VLPs, (Virus Like Particles)

STRUCTURE: Amino acids 1-381 of the pl protein encoded by the yeast retrotransposon Ty, followed by a unique restriction site for the insertion of foreign sequences and a translational stop codon. Sequence of the p1 Ty protein:
MESQQLSQHSPISHGSACASVTSKEVHTNQDPLDVSASKTEECEKASTKANSQQTTPASSAVPENP
HHASPQTAQSHSPQNGPYPQQCMMTQNQANPSGWSFYGHPSMIPYTPYQMSPMYFPPGPQSQFP
QYPSSVGTPLRT'PSPESGNTFTDSSSADSDMTSTKKYVRPPPMLTSPNDFPNWVKTYIKFLQNSNLG
GIIPTVNGKPVRQITDDELTFLYNTFQIFAPSQFLPTWVKDILSVDYTDIMKILSKSIEKMQSDTQEAN
DIVTLANLQYNGSTPADAFETKVTNIIDRLNNNGIHINNKVACQLIMRGLSGEYKFLRYTRHRHLN
MTVAELFLDIHAIYEEQQGSRNSKPNYRRNPSDEKNDSRSYTNTrKPKAGS K*

SOURCE: Recombinant protein produced from *Saccharomyces cerevisiae.* Purified by filtration and chromatography techniques.

USES: As a carrier protein for expressing foreign antigens. Hybrid Ty particles induce cell-mediated immunity (without additional adjuvant) and humoral immunity (with aluminum hydroxide).

APPEARANCE: Clear aqueous solution.

MOLECULAR WEIGHT: Monomer 42 kD

RECOMMENDED STORAGE: Store purified Ty particles at -20° C. Particles formulated with aluminum hydroxide should not be frozen, and can be stored at 4'C for 1-2 years.

CHEMICAL/PHYSICAL PROPERTIES: Approximately 300 monomers assemble to form a Ty particle.

INCOMPATIBILITY: Avoid contact with proteases.

SAFETY/TOXICITY: No systemic toxicity observed in human Phase I clinical trials (maximum dose 0.5 mg/subject, administered 4 times).

ADJUVANT PROPERTIES: Ty particles present antigen in a polyvalent, particulate form. Cytotoxic T-lymphocytes are induced in the absence of any other adjuvant formulations.

- Adams, S. E. *et al*., 1987, The expression of hybrid Ty virus-like particles in yeast. *Nature*, 329:68-70.
- Layton, G. T. *et al*., 1993, Induction of HIV-1 specific cytotoxic T-lymphocytes in vivo by immunization with hybrid HIV-1: Ty virus-like particles. *J. Immunol*., 151:1097-1107.
- Adams, S. and Kingsman, A., 1995, Retrovirus and retrotransposon particles as antigen presentation and delivery systems, in: Vaccine Design, M. F. Powell and M. J Newman (Eds.) Pharmaceutical Biotechnology Series, Plenum Publishing Corp., New York.

CONTACT(S): British BioTechnology Ltd., Oxford, OX4-5LY. Ph: 44-865-748747, Fax: 44-865-781-187.

COMPONENT/ADJUVANT NAME: Walter Reed Liposomes

OTHER NAME(S): Liposomes containing lipid A adsorbed to aluminum hydroxide, [L(Lipid A + Antigen) + Alum]

STRUCTURE: Phosphohpids: dimyristoyl phosphatidylchohne and dimyristoyl phosphatidylglycerol, cholesterol, Lipid A: from *Salmonella minnesota* R595, heterogeneous mixture of structures, molecular weight ranging from 1400-1800 depending on number of fatty acids and phosphate groups present. Generic structure of lipid A from *S.minnesota* R595: Aluminum hydroxide gel.

SOURCE: Phospholipids and cholesterol are obtained in pure form, GMP grade, from Avanti Polar Lipids, Inc. Native Lipid A, prepared by acid hydrolysis of the lipopolysaccharide of S. *minnesota* R595 is obtained from List Biological Laboratories. Monophosphoryl lipid A, a fraction purified from native lipid A, is obtained from Ribi ImmunoChem. Aluminum hydroxide gel is Alhydrogel or Rehydragel LV.

USES: Liposomes provide a vehicle for delivery of antigen to the immune system and also a mild adjuvant activity, but liposomes containing lipid A provide a very potent adjuvant activity. Adsorption of liposomes containing lipid A to aluminum hydroxide gel contributes additional strong adjuvant activity with many antigens. Liposomes containing lipid A have been shown to induce both humoral and cell-mediated immunity.

APPEARANCE: White opalescent particulate suspension.

MOLECULAR WEIGHT: Equal to the sum of the molecular weights of the components used in the formulation, e.g., antigen molecular weight will vary with the vaccine formulation.

RECOMMENDED STORAGE: Store liquid liposome formulations at 4-6° C. Lyophilized liposomes prior to reconstitution with antigen may be stored at either 4-6° C or -20° C. Liposomes in the liquid form reconstituted with antigen are stable for at least 1-2 years.

CHEMICAL/PHYSICAL PROPERTIES: Liposomes are stable at pH from 1 to 10. Solubility and stability will depend on the antigen encapsulated. Phospholipids, cholesterol, and native lipid A are soluble in chloroform. Monophosphoryl lipid A is soluble in chloroform-methanol 9:1. All liposomal components are stable in organic solvents for at least 1 year when stored at -20° C.

INCOMPATIBILITY: None found.

SAFETY/TOXICITY: Liposomal vaccine formulations have been administered to humans in four Phase I or Phase I/IIa clinical trials (three containing recombinant antigens derived from the *Plasmodium falciparum* sporozoite and one containing gp120 derived from the envelope of HIV). The vaccine formulations used in all four trials passed all preclinical safety and toxicity tests and no adverse side reactions have been observed.

Fries, L. F. *et al*., 1992, Liposomal malaria vaccine in humans: A safe and potent adjuvant strategy. *Proc. Natl. Acad. Sci*. USA, 89:358-362.

ADJUVANT PROPERTIES:

- Alving, C. R. and Richards, R. L., 1990, Liposomes containing lipid A: A potent nontoxic adjuvant for a human malaria sporozoite vaccine. *Immunol. Letters*, 25:275-280.

- Verma, J. N. *et al*., 1992, Adjuvant effects of liposomes containing lipid A: Enhancement of liposomal antigen presentation and recruitment of macrophages. *Infect. Immun*., 60:2438-2444.
- Alving, C. R. *et al*., 1992, Liposomes containing lipid A as a potent non-toxic adjuvant. *Res. Immunol*., 143:249-251.
- Alving, C. R. *et al*., 1993, Novel adjuvant strategies for experimental malaria and AIDS vaccines. Ann. N.Y. Acad. Sci., 690:265-275.

CONTACT(S): Dr. Carl Alving, Department of Membrane Biochemistry, Walter Reed Army Institute of Research,

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